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<input type="checkbox"/>	L15	(anti-nucleolin)same(p7-1A4)	3
<input type="checkbox"/>	L14	(anti-PARP)same(sc-1562)same(sc8007)same(sc1561)same(sc7150)	0
<input type="checkbox"/>	L13	L11 and anti-PARP	5
<input type="checkbox"/>	L12	L11 and anti-nucleolin	0
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<input type="checkbox"/>	L9	(bates)adj(paula)	7
<input type="checkbox"/>	L8	L7 and PARP	22
<input type="checkbox"/>	L7	L6 and apoptosis	139
<input type="checkbox"/>	L6	L5 and antibody	318
<input type="checkbox"/>	L5	nucleolin	350
<input type="checkbox"/>	L4	L1 and anti-nucleolin	2
<input type="checkbox"/>	L3	L2 and nucleolin	0
<input type="checkbox"/>	L2	L1 and anti-PARP	51
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=> s detection

L1 2026423 DETECTION

=> s l1 and apoptosis

L2 17620 L1 AND APOPTOSIS

=> s l2 and anti-nucleolin

L3 2 L2 AND ANTI-NUCLEOLIN

=> dup remove l3

PROCESSING COMPLETED FOR L3

L4 2 DUP REMOVE L3 (0 DUPLICATES REMOVED)

=> d l4 1-2 chib abs

L4 ANSWER 1 OF 2 CAPLUS COPYRIGHT 2005 ACS on STN

2004:20982 Document No. 140:90312 A method for the **detection** of **apoptosis** via determination of nucleolin and/or PARP-1 in the sample. Bates, Paula J.; Mi, Yingchang (University of Louisville Research Foundation, Inc., USA). PCT Int. Appl. WO 2004003554 A1 20040108, 66 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, UZ, VC, VN, YU, ZA, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2003-US20167 20030626. PRIORITY: US 2002-2002/PV392143 20020626.

AB Methods for the **detection** of **apoptosis** by measuring apoptotic bodies shed by apoptotic cells are provided, as are kits to carry out such methods. The method comprises preparing a sample from which cells have been removed and determining at least one of nucleolin and PARP-1 in the sample. The **detection** of either (or both) compds. comprises the **detection** of a nucleolin (PARP-1)-binding mol.-nucleolin (PARP-1) complex. The binding mols. are **anti-nucleolin** (anti-PARP-1) antibodies and for nucleolin also guanosine-rich oligonucleotides. The sample can be blood, serum, plasma, tissue, tissue culture medium, or sputum. The method can be used to determine excessive **apoptosis** via preparing a blood sample from a subject suspected of having a disease selected from the group consisting of AIDS, neurodegenerative disease, ischemic injury, autoimmune disease, tumor, cancer, viral infection, acute inflammation, and sepsis.

L4 ANSWER 2 OF 2 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN

2002:271588 Document No.: PREV200200271588. Nucleolin is a calcium-binding protein. Gilchrist, James S. C. [Reprint author]; Abrenica, Bernard; DiMario, Patrick J.; Czubryt, Michael P.; Pierce, Grant N.. Division of Stroke and Vascular Disease, St. Boniface General Hospital Research

Centre, 351 Tache Avenue, Room 4024, Winnipeg, Manitoba, R2H 2A6, Canada.
Journal of Cellular Biochemistry, (2002) Vol. 85, No. 2, pp. 268-278.
print.

CODEN: JCEBD5. ISSN: 0730-2312. Language: English.

AB We have purified a prominent 110-kDa protein (p110) from 1.6 M NaCl extracts of rat liver nuclei that appears to bind Ca^{2+} . p110 was originally identified by prominent blue staining with 'Stains-All' in sodium dodecyl sulfate-polyacrylamide gels and was observed to specifically bind ruthenium red and $^{45}\text{Ca}^{2+}$ in nitrocellulose blot overlays. In spin-dialysis studies, purified p110 saturably bound approximately 75 nmol Ca^{2+} /mg protein at a concentration of 1 mM total Ca^{2+} with half-maximal binding observed at 105 μM Ca^{2+} . With purification, p110 became increasingly susceptible to proteolytic (likely autolytic) fragmentation, although most intermediary peptides between 40 and 90 kDa retained "Stains-All", ruthenium red, and $^{45}\text{Ca}^{2+}$ binding. N-terminal sequencing of intact p110 and a 70-kDa autolytic peptide fragment revealed a strong homology to nucleolin. Two-dimensional sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE)/IEF revealed autolysis produced increasingly acidic peptide fragments ranging in apparent pI's from 5.5 for intact p110 to 3.5 for a 40 kDa peptide fragment. Intact p110 and several peptide fragments were immunostained with a highly specific anti-nucleolin antibody, R2D2, thus confirming the identity of this protein with nucleolin. These annexin-like Ca^{2+} -binding characteristics of nucleolin are likely contributed by its highly acidic argyrophilic N-terminus with autolysis apparently resulting in largely selective removal of its basic C-terminal domain. Although the Ca^{2+} -dependent functions of nucleolin are unknown, we discuss the possibility that like the structurally analogous HMG-1, its Ca^{2+} -dependent actions may regulate chromatin structure, possibly during **apoptosis**.

=> s l3 and anti-PARP-1
L5 1 L3 AND ANTI-PARP-1

=> d l5 cbib abs

L5 ANSWER 1 OF 1 CAPLUS COPYRIGHT 2005 ACS on STN
2004:20982 Document No. 140:90312 A method for the **detection of apoptosis** via determination of nucleolin and/or PARP-1 in the sample. Bates, Paula J.; Mi, Yingchang (University of Louisville Research Foundation, Inc., USA). PCT Int. Appl. WO 2004003554 A1 20040108, 66 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, UZ, VC, VN, YU, ZA, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2003-US20167 20030626. PRIORITY: US 2002-2002/PV392143 20020626.

AB Methods for the **detection of apoptosis** by measuring apoptotic bodies shed by apoptotic cells are provided, as are kits to carry out such methods. The method comprises preparing a sample from which cells have been removed and determining at least one of nucleolin and PARP-1 in the sample. The **detection** of either (or both) compds. comprises the **detection** of a nucleolin (PARP-1)-binding mol.-nucleolin (PARP-1) complex. The binding mols. are **anti-nucleolin (anti-PARP-1)** antibodies and for nucleolin also guanosine-rich oligonucleotides. The sample can be blood, serum, plasma, tissue, tissue culture medium, or sputum. The method can be used to determine excessive **apoptosis** via preparing a blood sample from a subject suspected of having a disease selected from the group consisting of AIDS, neurodegenerative disease, ischemic injury, autoimmune disease,

tumor, cancer, viral infection, acute inflammation, and sepsis.

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L1 2026423 S DETECTION
L2 17620 S L1 AND APOPTOSIS
L3 2 S L2 AND ANTI-NUCLEOLIN
L4 2 DUP REMOVE L3 (0 DUPLICATES REMOVED)
L5 1 S L3 AND ANTI-PARP-1

=> s l2 and nucleolin

L6 7 L2 AND NUCLEOLIN

=> dup remove l6

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L7 7 DUP REMOVE L6 (0 DUPLICATES REMOVED)

=> d l7 1-7 cbib abs

L7 ANSWER 1 OF 7 CAPLUS COPYRIGHT 2005 ACS on STM
2004:20982 Document No. 140:90312 A method for the **detection** of **apoptosis** via determination of **nucleolin** and/or PARP-1 in the sample. Bates, Paula J.; Mi, Yingchang (University of Louisville Research Foundation, Inc., USA). PCT Int. Appl. WO 2004003554 A1 20040108, 66 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, UZ, VC, VN, YU, ZA, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2003-US20167 20030626. PRIORITY: US 2002-2002/PV392143 20020626.

AB Methods for the **detection** of **apoptosis** by measuring apoptotic bodies shed by apoptotic cells are provided, as are kits to carry out such methods. The method comprises preparing a sample from which cells have been removed and determining at least one of **nucleolin** and PARP-1 in the sample. The **detection** of either (or both) compds. comprises the **detection** of a **nucleolin** (PARP-1)-binding mol.-**nucleolin** (PARP-1) complex. The binding mols. are anti-**nucleolin** (anti-PARP-1) antibodies and for **nucleolin** also guanosine-rich oligonucleotides. The sample can be blood, serum, plasma, tissue, tissue culture medium, or sputum. The method can be used to determine excessive **apoptosis** via preparing a blood sample from a subject suspected of having a disease selected from the group consisting of AIDS, neurodegenerative disease, ischemic injury, autoimmune disease, tumor, cancer, viral infection, acute inflammation, and sepsis.

L7 ANSWER 2 OF 7 CAPLUS COPYRIGHT 2005 ACS on STM
2002:937303 Document No. 138:20443 Endocrine disruptor screening using DNA chips of endocrine disruptor-responsive genes. Kondo, Akihiro; Takeda, Takeshi; Mizutani, Shigetoshi; Tsujimoto, Yoshimasa; Takashima, Ryokichi; Enoki, Yuki; Kato, Ikunoshin (Takara Bio Inc., Japan). Jpn. Kokai Tokkyo Koho JP 2002355079 A2 20021210, 386 pp. (Japanese). CODEN: JKXXAF. APPLICATION: JP 2002-69354 20020313. PRIORITY: JP 2001-73183 20010314; JP 2001-74993 20010315; JP 2001-102519 20010330.

AB A method and kit for detecting endocrine-disrupting chems. using DNA microarrays are claimed. The method comprises preparing a nucleic acid

sample containing mRNAs or cDNAs originating in cells, tissues, or organisms which have been brought into contact with a sample containing the endocrine disruptor. The nucleic acid sample is hybridized with DNA microarrays having genes affected by the endocrine disruptor or DNA fragments originating in these genes have been fixed. The results obtained are then compared with the results obtained with the control sample to select the gene affected by the endocrine disruptor. Genes whose expression is altered by tri-Bu tin, 4-octaphenol, 4-nonylphenol, di-N-Bu phthalate, dichlorohexyl phthalate, octachlorostyrene, benzophenone, diethylhexyl phthalate, diethylstilbestrol (DES), and 17- β estradiol (E2), were found in mice by DNA chip anal.

L7 ANSWER 3 OF 7 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN 2002:271588 Document No.: PREV200200271588. **Nucleolin** is a calcium-binding protein. Gilchrist, James S. C. [Reprint author]; Abrenica, Bernard; DiMario, Patrick J.; Czubyrt, Michael P.; Pierce, Grant N.. Division of Stroke and Vascular Disease, St. Boniface General Hospital Research Centre, 351 Tache Avenue, Room 4024, Winnipeg, Manitoba, R2H 2A6, Canada. Journal of Cellular Biochemistry, (2002) Vol. 85, No. 2, pp. 268-278. print.

CODEN: JCEBD5. ISSN: 0730-2312. Language: English.

AB We have purified a prominent 110-kDa protein (p110) from 1.6 M NaCl extracts of rat liver nuclei that appears to bind Ca^{2+} . p110 was originally identified by prominent blue staining with 'Stains-All' in sodium dodecyl sulfate-polyacrylamide gels and was observed to specifically bind ruthenium red and $^{45}\text{Ca}^{2+}$ in nitrocellulose blot overlays. In spin-dialysis studies, purified p110 saturably bound approximately 75 nmol Ca^{2+} /mg protein at a concentration of 1 mM total Ca^{2+} with half-maximal binding observed at 105 μM Ca^{2+} . With purification, p110 became increasingly susceptible to proteolytic (likely autolytic) fragmentation, although most intermediary peptides between 40 and 90 kDa retained "Stains-All", ruthenium red, and $^{45}\text{Ca}^{2+}$ binding. N-terminal sequencing of intact p110 and a 70-kDa autolytic peptide fragment revealed a strong homology to **nucleolin**. Two-dimensional sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE)/IEF revealed autolysis produced increasingly acidic peptide fragments ranging in apparent pI's from 5.5 for intact p110 to 3.5 for a 40 kDa peptide fragment. Intact p110 and several peptide fragments were immunostained with a highly specific anti-**nucleolin** antibody, R2D2, thus confirming the identity of this protein with **nucleolin**. These annexin-like Ca^{2+} -binding characteristics of **nucleolin** are likely contributed by its highly acidic argyrophilic N-terminus with autolysis apparently resulting in largely selective removal of its basic C-terminal domain. Although the Ca^{2+} -dependent functions of **nucleolin** are unknown, we discuss the possibility that like the structurally analogous HMG-1, its Ca^{2+} -dependent actions may regulate chromatin structure, possibly during **apoptosis**.

L7 ANSWER 4 OF 7 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN 2001:189491 Document No.: PREV200100189491. Alteration of argyrophilic nucleolar organizer region associated (Ag-NOR) proteins in **apoptosis**-induced human salivary gland cells and human oral squamous carcinoma cells. Morimoto, Yasuhiro; Kito, Shinji; Ohba, Takeshi; Morimoto, Hiroyuki; Okamura, Hirohiko; Haneji, Tatsuji [Reprint author]. Department of Histology and Oral Histology, School of Dentistry, The University of Tokushima, 18-15, 3 Kuramoto-cho, Tokushima, 770-8504, Japan. Journal of Oral Pathology and Medicine, (April, 2001) Vol. 30, No. 4, pp. 193-199. print.

ISSN: 0904-2512. Language: English.

AB The level of argyrophilic nucleolar organizer regions (AgNORs) and AgNOR-associated proteins (Ag-NOR proteins) varies with cell activity, including ribosomal biogenesis occurring in proliferating cells. Proteins associated with some AgNORs are detected by a specific silver staining. To investigate a possible relationship between **apoptosis** and the AgNORs or Ag-NOR proteins, we examined the changes of AgNORs and Ag-NOR

proteins during **apoptosis** in a human salivary gland cell line, HSG cells, and a human oral squamous carcinoma cell line, SCC-25 cells. **Apoptosis** was induced by treatment of HSG and SCC-25 cells with okadaic acid. Proteins prepared from HSG and SCC-25 cells treated with varying concentrations of okadaic acid (OA) were subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) followed by transferring to transfer membranes and staining for Ag-NOR proteins by modified Western blot analysis. Four major bands (110 kDa, 43 kDa, 39kDa, and 37 kDa) were detected in the proteins obtained from the control cells. The level of the 110-kDa protein decreased in the proteins prepared from OA-induced apoptotic cells; however, the reaction intensity of the other three bands was changed in apoptotic cells. An additional band of an 80-kDa Ag-NOR protein appeared and increased in the apoptotic cells. Cellular fractionation of HSG cells and SCC-25 cells was done with or without apoptotic induction. An 80-kDa Ag-NOR protein was detected in the nuclear fraction prepared from the apoptotic cells, while the 110-kDa protein decreased in the nuclear fraction of these cells. The 110-kDa Ag-NOR protein may be **nucleolin** (C23) as deduced from its AgNOR staining features, including molecular weight. The 80-kDa protein may be the cleavage product of the 110-kDa protein. In the cell-free apoptotic system, in which intact nuclei of HSG cells were incubated with the cytosol fraction of apoptotic HSG and SCC-25 cells, the 80-kDa Ag-NOR protein was detected in nuclei incubated with the cytosol fraction of apoptotic cells, while the level of the 110-kDa protein decreased. The changes of Ag-NOR proteins in nuclei prepared from SCC-25 cells incubated with cytosol fractions prepared from HSG and SCC-25 cells were identical to those of the HSG cells. The alternation of AgNORs in **apoptosis**-induced HSG cells was also examined using double staining with Hoechst 33342 and silver nitrate. Hoechst staining revealed typical apoptotic nuclei, which exhibited highly fluorescent condensed chromatin in OA-treated HSG cells. Silver grains representing AgNORs were not detected in the cells undergoing **apoptosis**. The dual-imposition view confirmed that AgNORs, which are visible as dots in nucleoli in the control cells, disappeared from the apoptotic nuclei of HSG cells. Our results indicate that the 110-kDa nucleolar Ag-NOR protein is associated with **apoptosis** and is cleaved during **apoptosis**.

L7 ANSWER 5 OF 7 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN 2000:335747 Document No.: PREV200000335747. Behavior of nucleolar proteins during the course of **apoptosis** in camptothecin-treated HL60 cells. Martelli, Alberto M. [Reprint author]; Robuffo, Iole; Bortul, Roberta; Ochs, Robert L.; Luchetti, Francesca; Cocco, Lucio; Zwyer, Marina; Bareggi, Renato; Falcieri, Elisabetta. Dipartimento di Morfologia Umana Normale, Universita di Trieste, via Manzoni 16, 34138, Trieste, Italy. Journal of Cellular Biochemistry, (May, 2000) Vol. 78, No. 2, pp. 264-277. print.

CODEN: JCEBD5. ISSN: 0730-2312. Language: English.

AB By means of immunofluorescence and immunoelectron microscopy we have studied the fate of different nucleolar components during the apoptotic process in camptothecin-treated HL60 cells. We have found that RNA polymerase 1 disappeared while UBF was associated with previously described fibrogranular threaded bodies. In contrast, fibrillarin, C23/**nucleolin**, and B23/nucleophosmin remained detectable in granular material present amid micronuclei of late apoptotic cells. Double immunolabeling experiments showed colocalization of both C23 and B23 with fibrillarin. Immunoblotting analysis showed that UBF was proteolytically degraded, whereas fibrillarin, C23/**nucleolin**, and B23/nucleophosmin were not. These results may help explain the presence of anti-nucleolar antibodies seen in various pathological disorders.

L7 ANSWER 6 OF 7 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN 1999:140898 Document No.: PREV199900140898. Parathyroid hormone-related protein interacts with RNA. Aarts, Michelle M.; Levy, David; He, Bin; Stregger, Shelley; Chen, Taiping; Richard, Stephane; Henderson, Janet E. [Reprint author]. Lady Davis Inst., Rm. 602, 3755 Cote Ste. Catherine Rd.,

Montreal, QC H3T 1E2, Canada. Journal of Biological Chemistry, (Feb. 19, 1999) Vol. 274, No. 8, pp. 4832-4838. print.

CODEN: JBCHA3. ISSN: 0021-9258. Language: English.

- AB Parathyroid hormone-related protein (PTHrP) is a secreted protein that acts as an autocrine and paracrine mediator of cell proliferation and differentiation. In addition to its biological activity that is mediated through signal transduction cascades, there is evidence for an intracellular role for PTHrP in cell cycle progression and **apoptosis**. These effects are mediated through a mid-region nuclear targeting sequence (NTS) that localizes PTHrP to the region of the nucleolus where ribonucleoprotein complexes form in vivo. In this work, we show that endogenous, transfected, and in vitro translated PTHrP proteins bind homopolymeric and total cellular RNAs at salt concentrations up to 1 M. A peptide representing the PTHrP NTS was effective in competing with the wild-type protein for RNA binding, whereas a similar peptide representing the **nucleolin** NTS was not. Site-directed mutagenesis revealed that the binding of PTHrP to RNA was direct and was dependent on preservation of a core GXKKXXK motif, embedded in the PTHrP NTS, which is shared with other RNA-binding proteins. The current observations are the first to document RNA binding by a secreted cellular protein and predict a role for PTHrP in regulating RNA metabolism that may be related to its localization in the nucleolus of cells in vivo.

L7 ANSWER 7 OF 7 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN 1998:505717 Document No.: PREV199800505717. Identification of **apoptosis**-associated proteins in a human Burkitt lymphoma cell line. Cleavage of heterogeneous nuclear ribonucleoprotein A1 by caspase 3. Brockstedt, Ekkehard; Rickers, Anke; Kostka, Susanne; Laubersheimer, Andreas; Doerken, Bernd; Wittmann-Liebold, Brigitte [Reprint author]; Bommert, Kurt; Otto, Albrecht. Proteinchemie, Max-Delbrueck-Centrum Mol. Med., Robert-Roessle-Strasse 10, D-72076 Tuebingen, Germany. Journal of Biological Chemistry, (Oct. 23, 1998) Vol. 273, No. 43, pp. 28057-28064. print.

CODEN: JBCHA3. ISSN: 0021-9258. Language: English.

- AB **Apoptosis** or programmed cell death is essential in the process of controlling lymphocyte growth and selection. We identified proteins that are involved in anti-IgM antibody-mediated **apoptosis** using a subclone of the human Burkitt lymphoma cell line BL60. **Apoptosis**-associated proteins were detected by high resolution two-dimensional gel electrophoresis on a micropreparative scale. Comparison of the high resolution two-dimensional gel electrophoresis protein patterns from apoptotic and non-apoptotic cells showed differences in approx 80 spots including protein modifications. Analysis of the predominantly altered proteins was performed by internal Edman microsequencing and/or by peptide mass fingerprinting using matrix-assisted laser desorption/ionization mass spectrometry. Analysis was significantly improved by using new micropreparative high resolution two-dimensional gels employing high protein concentrations. The following 12 **apoptosis**-associated proteins were identified: heterogeneous nuclear ribonucleoprotein (hnRNP) A1, hnRNP C1/C2, FUSE-binding protein, dUTPase, lymphocyte-specific protein LSP1, UV excision repair protein RAD23 homologue B (HHR23B), 60 S acidic ribosomal protein P0 (L10E), heterochromatin protein 1 homologue alpha (HP1alpha), **nucleolin**, lamin, neutral calponin, and actin. Fragmentation of actin, hnRNP A1, hnRNP C1/C2, 60 S acidic ribosomal protein P0, lamin, and **nucleolin** could be inhibited by benzyloxycarbonyl-Asp(OMe)-Glu(OMe)-Val-Asp(OMe)fluoromethyl ketone, a selective irreversible inhibitor of CPP32 (caspase 3).

=> s 12 and "PARP-1"

L8 43 L2 AND "PARP-1"

=> dup remove 18

PROCESSING COMPLETED FOR L8

L9 17 DUP REMOVE L8 (26 DUPLICATES REMOVED)

=> d l9 1-17 cbib abs

L9 ANSWER 1 OF 17 CAPLUS COPYRIGHT 2005 ACS on STN

2005:572456 Document No. 143:71726 Methods of identifying anti-cancer agents and uses thereof. Thompson, Craig B.; Zong, Wei-Xing (USA). U.S. Pat. Appl. Publ. US 2005142621 A1 20050630, 23 pp. (English). CODEN: USXXCO. APPLICATION: US 2004-13574 20041215. PRIORITY: US 2003-2003/PV529642 20031215.

AB The invention provides methods of identifying and/or detecting anti-cancer agents. The invention provides methods of identifying and/or detecting compds. that can activate PARP and/or induce necrosis. The invention also provides for methods of treating cancer in an individual. The invention also provides kits for identifying and/or detecting anti-cancer agents.

L9 ANSWER 2 OF 17 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation on STN

2005:903935 The Genuine Article (R) Number: 960YW. Poly(ADP-ribosyl)ation by **PARP-1**: 'PAR-laying' NAD(+) into a nuclear signal. Kim M Y; Zhang T; Kraus W L (Reprint). Cornell Univ, Dept Mol Biol & Genet, Ithaca, NY 14853 USA (Reprint); Cornell Univ, Weill Med Coll, Dept Pharmacol, New York, NY 10021 USA. wlk5@cornell.edu. GENES & DEVELOPMENT (1 SEP 2005) Vol. 19, No. 17, pp. 1951-1967. ISSN: 0890-9369. Publisher: COLD SPRING HARBOR LAB PRESS, PUBLICATIONS DEPT, 500 SUNNYSIDE BLVD, WOODBURY, NY 11797-2924 USA. Language: English.
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Poly(ADP-ribose) (PAR) and the PAR polymerases (PARPs) that catalyze its synthesis from donor nicotinamide adenine dinucleotide (NAD(+)) molecules have received considerable attention in the recent literature. Poly(ADP-ribosyl)ation (PARylation) plays diverse roles in many molecular and cellular processes, including DNA damage **detection** and repair, chromatin modification, transcription, cell death pathways, insulator function, and mitotic apparatus function. These processes are critical for many physiological and pathophysiological outcomes, including genome maintenance, carcinogenesis, aging, inflammation, and neuronal function. This review highlights recent work on the biochemistry, molecular biology, physiology, and pathophysiology of PARylation, focusing on the activity of **PARP-1**, the most abundantly expressed member of a family of PARP proteins. In addition, connections between nuclear NAD' metabolism and nuclear signaling through **PARP-1** are discussed.

L9 ANSWER 3 OF 17 MEDLINE on STN DUPLICATE 1

2005084401. PubMed ID: 15523000. Poly(ADP-Ribose) polymerase promotes cardiac remodeling, contractile failure, and translocation of **apoptosis**-inducing factor in a murine experimental model of aortic banding and heart failure. Xiao Chun-Yang; Chen Min; Zsengeller Zsuzsanna; Li Hongshan; Kiss Levente; Kollai Mark; Szabo Csaba. (Inotek Pharmaceuticals Corporation, Suite 419E, 100 Cummings Center, Beverly, MA 01915, USA.) Journal of pharmacology and experimental therapeutics, (2005 Mar) 312 (3) 891-8. Electronic Publication: 2004-11-02. Journal code: 0376362. ISSN: 0022-3565. Pub. country: United States. Language: English.

AB Oxidant stress-induced activation of poly(ADP-ribose) polymerase (PARP) plays a role in the pathogenesis of various cardiovascular diseases. We have now investigated the role of PARP in the process of cardiac remodeling and heart failure in a mouse model of heart failure induced by transverse aortic constriction (banding). The catalytic activity of PARP was inhibited by the potent isoindolinone-based PARP inhibitor INO-1001 or by **PARP-1** genetic deficiency. PARP inhibition prevented the pressure overload-induced decrease in cardiac contractile function, despite the pressure gradient between both carotid arteries being comparable in the two experimental groups. The development of hypertrophy, the formation of collagen in the hearts, and the mitochondrial-to-nuclear translocation of the cell death factor

apoptosis-inducing factor (AIF) were attenuated by PARP inhibition. The ability of the inhibitor to block the catalytic activity of PARP was confirmed by immunohistochemical **detection** of poly(ADP-ribose), the product of the enzyme in the heart. Plasma levels of INO-1001, as measured at the end of the experiments, were in the concentration range sufficient to block the oxidant-mediated activation of PARP in murine cardiac myocytes in vitro. Myocardial hypertrophy and AIF translocation was also reduced in **PARP-1**-deficient mice undergoing aortic banding, compared with their wild-type counterparts. Overall, the current results demonstrate the importance of poly(ADP-ribos)ylation in the pathogenesis of banding-induced heart failure.

L9 ANSWER 4 OF 17 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation on STN

2005:172402 The Genuine Article (R) Number: 896JX. **PARP-1** and **PARP-2** interact with nucleophosmin/beta 23 and accumulate in transcriptionally active nucleoli. Meder V S; Boeglin M; de Murcia G; Schreiber V (Reprint). Univ Strasbourg 1, CNRS, UPR 9003, Lab Conventione Commissariat Energie Atom, Ecole, Blvd Sebastien Brant, BP10413, F-67412 Illkirch Graffenstaden, France (Reprint); Univ Strasbourg 1, CNRS, UPR 9003, Lab Conventione Commissariat Energie Atom, Ecole, F-67412 Illkirch Graffenstaden, France; ULP, CNRS INSERM, Coll France, Inst Genet & Biol Mol & Cellulaire, F-67404 Illkirch Graffenstaden, France. schreibe@esbs.u-strasbg.fr. JOURNAL OF CELL SCIENCE (1 JAN 2005) Vol. 118, No. 1, pp. 211-222. ISSN: 0021-9533. Publisher: COMPANY OF BIOLOGISTS LTD, BIDDER BUILDING CAMBRIDGE COMMERCIAL PARK COWLEY RD, CAMBRIDGE CB4 4DL, CAMBS, ENGLAND. Language: English.

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB The DNA damage-dependent poly(ADP-ribose) polymerases-1 and -2 (**PARP-1** and **PARP-2**) are survival factors that share overlapping functions in the **detection**, signaling and repair of DNA strand breaks resulting from genotoxic lesions in mammalian cells. Here we show that **PARP-1** and **PARP-2** subnuclear distributions partially overlap, with both proteins accumulating within the nucleolus independently of each other. **PARP-2** is enriched within the whole nucleolus and partially colocalizes with the nucleolar factor nucleophosmin/B23. We have identified a nuclear localization signal and a nucleolar localization signal within the N-terminal domain of **PARP-2**. **PARP-2**, like **PARP-1**, interacts with B23 through its N-terminal DNA binding domain. This association is constitutive and does not depend on either PARP activity the nucleolar localization signal of **PARP-2**. **PARP-1** and **PARP-2**, together with B23, are delocalized from the nucleolus upon RNA polymerase I inhibition whereas the nucleolar accumulation of all three proteins is only moderately affected upon oxidative or alkylated DNA damage. Finally, we show that murine fibroblasts deficient in **PARP-1** or **PARP-2** are not affected in the transcription of ribosomal RNAs. Taken together, these results suggest that the biological role of **PARP-1** and **PARP-2** within the nucleolus relies on functional nucleolar transcription, without any obvious implication of either PARP on this major nucleolar process.

L9 ANSWER 5 OF 17 CAPLUS COPYRIGHT 2005 ACS on STN

2005:79630 Document No. 142:293523 Direct quantitation of poly(ADP-ribose) polymerase (PARP) activity as a means to distinguish necrotic and apoptotic death in cell and tissue samples. Putt, Karson S.; Beilman, Gregory J.; Hergenrother, Paul J. (Department of Biochemistry, University of Illinois, Urbana, IL, 61801, USA). ChemBioChem, 6(1), 53-55 (English) 2005. CODEN: CBCHFX. ISSN: 1439-4227. Publisher: Wiley-VCH Verlag GmbH & Co. KGaA.

AB The enzyme poly(ADP-ribose) polymerase-1 (**PARP-1**) is differentially processed in **apoptosis** and necrosis, and therefore its activity can potentially be used as a means of distinguishing these two forms of cell death. In cells that die by

apoptosis, **PARP-1** is cleaved and inactivated, while during necrotic cell death, **PARP-1** is highly activated. The application of a **PARP-1** enzymic assay to the direct quantitation of **PARP** activity from cellular lysates allowing rapid and convenient **detection** and differentiation of apoptotic and necrotic cell death is demonstrated. This assay requires no specialized reagents and is also effective in whole animal tissue, indicating that it might be useful as a research or clin. diagnostic tool.

L9 ANSWER 6 OF 17 MEDLINE on STN DUPLICATE 2
2005190431. PubMed ID: 15823794. Multicolor fluorescence technique to detect apoptotic cells in advanced coronary atherosclerotic plaques. Soldani C; Scovassi A I; Canosi U; Bramucci E; Ardissino D; Arbustini E. (Laboratorio di Biologia Cellulare e Neurobiologia, Dipartimento di Biologia Animale, Universita di Pavia, Piazza Botta 10, 27100 Pavia, Italy.. soldani@unipv.it) . European journal of histochemistry : EJH, (2005 Jan-Mar) 49 (1) 47-52. Journal code: 9207930. ISSN: 1121-760X. Pub. country: Italy. Language: English.

AB **Apoptosis** occurring in atherosclerotic lesions has been suggested to be involved in the evolution and the structural stability of the plaques. It is still a matter of debate whether **apoptosis** mainly involves vascular smooth muscle cells (vSMCs) in the fibrous tissue or inflammatory (namely foam) cells, thus preferentially affecting the cell-poor lipid core of the atherosclerotic plaques. The aim of the present investigation was to detect the presence of apoptotic cells and to estimate their percentage in a series of atherosclerotic plaques obtained either by autopsy or during surgical atherectomy. Apoptotic cells were identified on paraffin-embedded sections on the basis of cell nuclear morphology after DNA staining and/or by cytochemical reactions (TUNEL assay, immunodetection of the proteolytic poly (ADP-ribose) polymerase-1 [**PARP-1**] fragment); biochemical procedures (identifying DNA fragmentation or **PARP-1** proteolysis) were also used. Indirect immunofluorescence techniques were performed to label specific antigens for either vSMCs or macrophages (i.e., the cells which are most likely prone to **apoptosis** in atherosclerotic lesions): the proper selection of fluorochrome labeling allowed the simultaneous **detection** of the cell phenotype and the apoptotic characteristics, by multicolor fluorescence techniques. Apoptotic cells proved to be less than 5% of the whole cell population, in atherosclerotic plaque sections: this is, in fact, a too low cell fraction to be detected by widely used biochemical methods, such as agarose gel electrophoresis of low-molecular-weight DNA or Western-blot analysis of **PARP-1** degradation. Most apoptotic cells were of macrophage origin, and clustered in the tunica media, near or within the lipid-rich core; only a few TUNEL-positive cells were labeled for antigens specific for vSMCs. These results confirm that, among the cell populations in atherosclerotic plaques, macrophage foam-cells are preferentially involved in **apoptosis**. Their death may decrease the cell number in the lipid core and generate a possibly defective apoptotic clearance: the resulting release of matrix-degrading enzymes could contribute to weakening the fibrous cap and promote the plaque rupture with the risk of acute ischemic events, while increasing the thrombogenic pultaceous pool of the plaque core.

L9 ANSWER 7 OF 17 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation on STN
2005:929581 The Genuine Article (R) Number: 962NM. A new multiplex assay allowing simultaneous **detection** of the inhibition of cell proliferation and induction of cell death. Wesierska-Gadek J (Reprint); Gueorguieva M; Ranftler C; Zerza-Schnitzhofer G. Med Univ Vienna, Inst Canc Res, Cell Cycle Regulat Grp, Dept Med 1, Borschkegasse 8A, A-1090 Vienna, Austria (Reprint); Med Univ Vienna, Inst Canc Res, Cell Cycle Regulat Grp, Dept Med 1, A-1090 Vienna, Austria; Tecan Austria GmbH, Grodeg, Austria. Jozefa.Gadek-Wesierski@meduniwien.ac.at. JOURNAL OF CELLULAR BIOCHEMISTRY (1 SEP 2005) Vol. 96, No. 1, pp. 1-7. ISSN:

0730-2312. Publisher: WILEY-LISS, DIV JOHN WILEY & SONS INC, 111 RIVER ST, HOBOKEN, NJ 07030 USA. Language: English.

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB The efficacy of distinct anti-cancer drugs used in the chemotherapy of human malignancies varies between tumor tissues and depends largely on the ability of the therapeutic agents to simultaneously inhibit cell proliferation and to eliminate malignant cells by **apoptosis**. Especially, **detection** of early apoptotic changes seems to be important because early stages of **apoptosis** differ from those of necrosis. Therefore, the development of a novel test allowing fast and concomitant screening of the anti-proliferative and pro-apoptotic action of a number of anti-cancer drugs is of great interest. For this purpose, we choose as an experimental model a well characterized anti-proliferative and pro-apoptotic effect of cisplatin (CP) on human cervical carcinoma HeLaS3 cells. As previously reported, exposure of HeLaS3 to CP resulted in a concomitant inhibition of cell proliferation and induction of **apoptosis** in a dose- and time-dependent manner. In the present study we performed two independent approaches. In the first approach, we examined the cell proliferation and activity of caspases-3/7 in two separate microtiter plates using the CellTiter-Glo (TM) Luminescent Cell Viability Assay and the Caspase-Glo (TM) 3/7 Assay, respectively. In the second approach, we determined the same parameters sequentially in one microtiter plate by a multiplexing assay using CellTiter-Blue (TM) Cell Viability Assay and Caspase-Glo (TM) 3/7 Assay. The both approaches gave very similar results indicating that this new multiplexing assay offers an important advantage for simultaneous **detection** of cell number and activation of caspases-3/7. The new multiplexing assay offers a range of benefits over standard assays.

L9 ANSWER 8 OF 17 CAPLUS COPYRIGHT 2005 ACS on STN

2004:20982 Document No. 140:90312 A method for the **detection** of **apoptosis** via determination of nucleolin and/or **PARP-1** in the sample. Bates, Paula J.; Mi, Yingchang (University of Louisville Research Foundation, Inc., USA). PCT Int. Appl. WO 2004003554 A1 20040108, 66 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, UZ, VC, VN, YU, ZA, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2003-US20167 20030626. PRIORITY: US 2002-2002/PV392143 20020626.

AB Methods for the **detection** of **apoptosis** by measuring apoptotic bodies shed by apoptotic cells are provided, as are kits to carry out such methods. The method comprises preparing a sample from which cells have been removed and determining at least one of nucleolin and **PARP-1** in the sample. The **detection** of either (or both) compds. comprises the **detection** of a nucleolin (**PARP-1**)-binding mol.-nucleolin (**PARP-1**) complex. The binding mols. are anti-nucleolin (anti-**PARP-1**) antibodies and for nucleolin also guanosine-rich oligonucleotides. The sample can be blood, serum, plasma, tissue, tissue culture medium, or sputum. The method can be used to determine excessive **apoptosis** via preparing a blood sample from a subject suspected of having a disease selected from the group consisting of AIDS, neurodegenerative disease, ischemic injury, autoimmune disease, tumor, cancer, viral infection, acute inflammation, and sepsis.

L9 ANSWER 9 OF 17 MEDLINE on STN

2004577880. PubMed ID: 15554186. [Poly(ADP-ribose) polymerase-1 as a regulator of protein-nucleic acid interactions in the processes responding to genotoxic action]. Poli(ADP-ribozo)polimeraza-1-regulator belkovo-nukleinovykh vzaimodeistvii v protsessakh voznikaiushchikh pri

genotoksicheskom vozdeistvii. Sukhanova M V; Lavrik O I; Khodyreva S N. Molekuliarnaia biologii, (2004 Sep-Oct) 38 (5) 834-47. Ref: 80. Journal code: 0105454. ISSN: 0026-8984. Pub. country: Russia: Russian Federation. Language: Russian.

- AB Poly(ADP-ribose) polymerase-1 (**PARP-1**), nuclear protein of higher eukaryotes, specifically detects strand breaks in DNA. When bound to DNA strand breaks, **PARP-1** is activated and catalyzes synthesis of poly(ADP-ribose) covalently attached to the row of nuclear proteins, with the main acceptor being **PARP-1** itself. This protein participates in a majority of DNA dependent processes: repair, recombination; replication; cell death: **apoptosis** and necrosis. Poly(ADP-ribosyl)ation of proteins is considered as mechanism, which signals about DNA damage and modulate protein functioning in response to genotoxic impact. The main emphasis is made on the roles of **PARP-1** and poly(ADP-ribosyl)ation in base excision repair (BER), the process, which provides repair of DNA breaks. The main proposed functions of **PARP-1** in this process are: factor initiating assemblage of protein complex of BER; temporary protection of DNA ends; modulation of chromatin structure via poly(ADP-ribosyl)ation of histones; signaling function in **detection** of the levels of DNA damage in cell.

L9 ANSWER 10 OF 17 MEDLINE on STN DUPLICATE 3

2004402567. PubMed ID: 15307148. In vivo analysis reveals different apoptotic pathways in pre- and postmigratory cerebellar granule cells of rabbit. Lossi Laura; Gambino Graziana; Mioletti Silvia; Merighi Adalberto. (Department of Veterinary Morphophysiology, University of Turin, Via Leonardo da Vinci 44, 10095 Grugliasco (TO) Italy, UE.) Journal of neurobiology, (2004 Sep 15) 60 (4) 437-52. Journal code: 0213640. ISSN: 0022-3034. Pub. country: United States. Language: English.

- AB Naturally occurring neuronal death (NOND) has been described in the postnatal cerebellum of several species, mainly affecting the cerebellar granule cells (CGCs) by an apoptotic mechanism. However, little is known about the cellular pathway(s) of CGC **apoptosis** in vivo. By immunocytochemistry, in situ **detection** of fragmented DNA, electron microscopy, and Western blotting, we demonstrate here the existence of two different molecular mechanisms of **apoptosis** in the rabbit postnatal cerebellum. These two mechanisms affect CGCs at different stages of their maturation and migration. In the external granular layer, premigratory CGCs undergo **apoptosis** upon phosphorylation of checkpoint kinase 1 (Chk1), and hyperphosphorylation of retinoblastoma protein. In postmigratory CGCs within the internal granular layer, caspase 3 and to a lesser extent 7 and 9 are activated, eventually leading to poly-ADP-ribose polymerase-1 (**PARP-1**) cleavage and programmed cell death. We conclude that NOND of premigratory CGCs is linked to activation of DNA checkpoint and alteration of normal cell cycle, whereas in postmigratory CGCs **apoptosis** is, more classically, dependent upon caspase 3 activation.

L9 ANSWER 11 OF 17 MEDLINE on STN DUPLICATE 4

2004482019. PubMed ID: 15451183. **Apoptosis** induced by a cytopathic hepatitis A virus is dependent on caspase activation following ribosomal RNA degradation but occurs in the absence of 2'-5' oligoadenylate synthetase. Goswami Biswendu B; Kulka Michael; Ngo Diana; Cebula Thomas A. (Division of Molecular Biology, Office of Applied Research and Safety Assessment, Food and Drug Administration, HFS-025, OARSA, FDA, 8301 Muirkirk Road, Laurel, MD 20708, USA, . bgoswami@cfsan.fda.gov) . Antiviral research, (2004 Sep) 63 (3) 153-66. Journal code: 8109699. ISSN: 0166-3542. Pub. country: Netherlands. Language: English.

- AB We have presented previously evidence that the cytopathogenic 18f strain of hepatitis A virus (HAV) induced degradation of ribosomal RNA (rRNA) in infected cells [Arch. Virol. 148 (2003) 1275-1300]. In contrast, the non-cytopathogenic parent virus HM175 clone 1 had no effect on rRNA integrity. We present here data showing that rRNA degradation is followed

by **apoptosis** accompanied by characteristic DNA laddering in the cytoplasm of 18f infected cells. The DNA laddering coincided with the **detection** of caspase 3 and **PARP-1** cleavage and was dependent upon activation of the caspase pathway, since treatment with Z-VAD-FMK, a pan-caspase inhibitor, inhibited both events. RNase L mRNA was present in both virus-infected and uninfected cells. Messenger RNA for the interferon inducible enzyme 2'-5' oligoadenylate synthetase (2'-5' OAS), which polymerizes ATP into 2'-5' oligo adenyate (2-5A, the activator of RNase L) in the presence of double-stranded RNA, was not detected following virus infection. 2'-5' OAS mRNA was induced by treatment of the cells with interferon-beta (IFN-beta). IFN-beta mRNA was marginally induced following infection. However, phosphorylated STAT 1, a key regulator of interferon-stimulated gene transcription was not detected in virus infected cells. STAT 1 phosphorylation in response to IFN treatment was lower in virus-infected cells, compared to uninfected cells treated with interferon, suggesting that 18f virus infection interferes with interferon signaling. The results suggest that 18f infection causes the induction of a 2-5A independent RNase L like activity.

L9 ANSWER 12 OF 17 MEDLINE on STN DUPLICATE 5
 2003182241. PubMed ID: 12625816. The onset of **apoptosis** of neurons induced by ischemia-reperfusion injury is delayed by transient period of hypertension in rats. Smrcka M; Horky M; Otevrel F; Kuchtickova S; Kotala V; Muzik J. (Institute of Pathophysiology, Medical School, Masaryk University, Brno, Czech Republic.) Physiological research / Academia Scientiarum Bohemoslovaca, (2003) 52 (1) 117-22. Journal code: 9112413. ISSN: 0862-8408. Pub. country: Czech Republic. Language: English.

AB We investigated the potential neuroprotective effect of transient hypertension on neuronal cell death induced by ischemia-reperfusion. Recovery of neurons, terminally differentiated cells, is almost entirely dependent upon active transcription and repair of DNA damage. We focused on the histochemical **detection** of distribution of NOR (argyrophilic nucleolar proteins) reflecting nucleolar integrity, immunohistochemical **detection** of **PARP-1** (poly(ADP-ribose) polymerase-1), MADD (mitogen-activated death domain), a protein accumulated in nucleoli upon stimulation by ischemia, the active form of caspase-3, a universal proteolytic enzyme of **apoptosis**. The terminal deoxynucleotidyl-transferase (TdT)-mediated dUTP-biotin nick-end-labeling method (TUNEL) proved the presence of in situ DNA fragmentation. We used the model of transient focal cerebral ischemia in rats with occlusion of middle cerebral artery. In experimental group of rats, the transient hypertension was induced by constriction of the abdominal aorta. The period of ischemia lasted 15, 30, 60 and 120 min followed by 48 h of reperfusion. We examined the frontal lobe of the ipsilateral hemisphere for **apoptosis** of neurons and compared it with the intact brain tissue. In normotensive rats with transient focal cerebral ischemia, we found disintegrated nucleoli of cortical as well as subcortical neurons at all investigated periods of ischemia, whereas the neurons of intact animals showed compact nucleoli with a few satellites. Nuclear positivity for MADD and **PARP-1** was apparent in the neocortex after 15 min and peaked after 30 min of ischemia. On the other hand, the subcortical neurons showed nuclear positivity after 60 and 120 min. The immunohistochemical reaction for active caspase 3 was apparent after 30 min onwards predominantly in the cortex. The TUNEL staining was distinct after 60 and 120 min. In hypertensive rats, we found nucleolar disintegration, positivity for MADD, **PARP-1** and caspase 3 after 30 min cortically and subcortically, followed by TUNEL positive staining of cortical neurons after 60 and 120 min. In summary, we detected delayed activation of neuronal **apoptosis** in transiently hypertensive rats with focal cerebral ischemia compared to normotensive animals. The apoptotic phenotype was confirmed by a panel of complementary methods showing rapid proteolysis-nucleolar segregation, MADD, **PARP-1** and caspase-3 positivity as well as ultimate DNA fragmentation proved by the TUNEL assay.

L9 ANSWER 13 OF 17 MEDLINE on STN DUPLICATE 6
2002122378. PubMed ID: 11857494. **Apoptosis** in B-cell lymphomas and reactive lymphoid tissues always involves activation of caspase 3 as determined by a new in situ **detection** method. Dukers Danny F; Oudejans Joost J; Vos Wim; ten Berge Rosita L; Meijer Chris J L M. (Department of Pathology, VU Medical Centre Amsterdam, De Boelelaan 1117, 1081 HV Amsterdam, The Netherlands.) Journal of pathology, (2002 Mar) 196 (3) 307-15. Journal code: 0204634. ISSN: 0022-3417. Pub. country: England: United Kingdom. Language: English.

AB In vitro studies indicate that in lymphomas, execution of **apoptosis** involves activation of effector caspases. To investigate activation of effector caspases in vivo in biopsy specimens of lymphomas, a new assay was developed using antibodies against active caspase 3 and p89, a protein fragment generated by caspase-specific cleavage of poly-ADP ribose polymerase (PARP). Using this assay, it was found that in B-cell lymphomas, levels of active caspase 3/p89-positive cells correlate strongly with morphologically recognizable apoptotic cells. The number of active caspase 3/p89-positive cells was low in follicular lymphomas and usually high in diffuse large cell lymphomas. Highest numbers were found in Burkitt lymphomas and in two biopsies of diffuse large B-cell lymphomas (DLCLs) obtained several days after initiation of therapy. It is concluded that **apoptosis** in reactive lymphoid tissues and in B-cell lymphomas always involves activation of effector caspase 3 and cleavage of one of the major effector caspase substrates, **PARP-1**. Moreover, levels of effector caspase activation are constantly low in low-grade follicular lymphomas and vary considerably in DLCL and Burkitt lymphoma. Copyright 2002 John Wiley & Sons, Ltd.

L9 ANSWER 14 OF 17 MEDLINE on STN DUPLICATE 7
2002327123. PubMed ID: 12070005. High numbers of active caspase 3-positive Reed-Sternberg cells in pretreatment biopsy specimens of patients with Hodgkin disease predict favorable clinical outcome. Dukers Danny F; Meijer Chris J L M; ten Berge Rosita L; Vos Wim; Ossenkoppele Gert J; Oudejans Joost J. (Department of Pathology, VU Medical Centre, Amsterdam, The Netherlands.) Blood, (2002 Jul 1) 100 (1) 36-42. Journal code: 7603509. ISSN: 0006-4971. Pub. country: United States. Language: English.

AB In vitro studies suggest that resistance to the **apoptosis** -inducing effect of chemotherapy might explain poor responses to therapy in fatal instances of Hodgkin disease (HD). Execution of **apoptosis** depends on proper functioning of effector caspases, in particular caspase 3, which is activated on the induction of **apoptosis** through either the stress-induced pathway or the death receptor-mediated pathway. Thus, high levels of caspase 3 activation should reflect proper functioning of one or both identified **apoptosis** pathways, resulting in chemotherapy-sensitive neoplastic cells and thus a favorable clinical response to chemotherapy. We tested this hypothesis by quantifying active caspase 3-positive tumor cells in primary biopsy specimens of HD and compared these numbers to clinical outcomes. Using an immunohistochemical assay, activation of caspase 3 was detected in 0% to 13% of neoplastic cells. High numbers of active caspase 3-positive tumor cells (5% or more) correlated with excellent clinical prognosis; 0 of 22 patients with 5% or more active caspase 3-positive cells died compared with 11 of 41 patients with less than 5% positive cells (P = .007). Proper functioning of active caspase 3 was demonstrated by the **detection** of one of its cleaved substrates, **PARP-1/p89**, in similar percentages of neoplastic cells. High levels of active caspase 3-positive neoplastic cells were associated with the expression of p53 and its downstream effector molecule p21, suggesting proper functioning of the stress-induced **apoptosis** pathway. In conclusion, high numbers of active caspase 3-positive neoplastic cells predict a highly favorable clinical outcome in HD patients, supporting the notion that an (at least partially) intact **apoptosis** cascade is essential for the cell killing effect of chemotherapy.

- L9 ANSWER 15 OF 17 MEDLINE on STN DUPLICATE 8
 2002114089. PubMed ID: 11846007. Two-color fluorescence **detection** of Poly (ADP-Ribose) Polymerase-1 (**PARP-1**) cleavage and DNA strand breaks in etoposide-induced apoptotic cells. Soldani C; Bottone M G; Pellicciari C; Scovassi A I. European journal of histochemistry : EJH, (2001) 45 (4) 389-92. Journal code: 9207930. ISSN: 1121-760X. Pub. country: Italy. Language: English.
- AB During **apoptosis**, the nuclear enzyme Poly(ADP-Ribose) Polymerase-1 (**PARP-1**) catalyzes the rapid and transient synthesis of poly(ADP-ribose) from NAD⁺ and becomes inactive when cleaved by caspases. The regulation of these two opposite roles of **PARP-1** is still unknown. We have recently investigated **PARP-1** activation/degradation in Hep-2 cells driven to **apoptosis** by actinomycin D. In the present work, we have extended our analysis to the effect of the DNA damaging agent etoposide, and paid attention to the relationship between **PARP-1** cleavage and DNA fragmentation. An original fluorescent procedure was developed to simultaneously identify in situ the p89 proteolytic fragment of **PARP-1** (by immunolabeling) and DNA degradation (by the TUNEL assay). The presence of p89 was observed both in cells with advanced signs of **apoptosis** (where the **PARP-1** fragment is extruded from the nucleus into the cytoplasm) and in TUNEL-negative cells, with only incipient signs of chromatin condensation; this evidence indicates that **PARP-1** degradation in etoposide-treated apoptotic cells may precede DNA cleavage.
- L9 ANSWER 16 OF 17 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation on STN
 2002:6669 The Genuine Article (R) Number: 503UV. Two-color fluorescence **detection** of Poly(ADP-ribose) Polymerase-1 (**PARP-1**) cleavage and DNA strand breaks in etoposide-induced apoptotic cells. Soldani C; Bottone M G; Pellicciari C; Scovassi A I (Reprint). CNR, Ist Genet Biochim & Evoluzionist, Via Abbiategrasso 207, I-27100 Pavia, Italy (Reprint); CNR, Ist Genet Biochim & Evoluzionist, I-27100 Pavia, Italy; Univ Pavia, Dipartimento Biol Anim, I-27100 Pavia, Italy; Univ Pavia, CNR, Ctr Studio Istochim, I-27100 Pavia, Italy. EUROPEAN JOURNAL OF HISTOCHEMISTRY (2001) Vol. 45, No. 4, pp. 389-392. ISSN: 1121-760X. Publisher: LUIGI PONZIO E FIGLIO, VIA D DA CATALOGNA 1/3, 27100 PAVIA, ITALY. Language: English.
 ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS
- AB During **apoptosis**, the nuclear enzyme Poly(ADP-Ribose) Polymerase-1 (**PARP-1**) catalyzes the rapid and transient synthesis of poly(ADP-ribose) from NAD(+) and becomes inactive when cleaved by caspases. The regulation of these two opposite roles of **PARP-1** is still unknown. We have recently investigated **PARP-1** activation/degradation in Hep-2 cells driven to **apoptosis** by actinomycin D. In the present work, we have extended our analysis to the effect of the DNA damaging agent etoposide, and paid attention to the relationship between **PARP-1** cleavage and DNA fragmentation. An original fluorescent procedure was developed to simultaneously identify in sitar the p89 proteolytic fragment of **PARP-1** (by immunolabeling) and DNA degradation (by the TUNEL assay). The presence of p89 was observed both in cells with advanced signs of **apoptosis** (where the **PARP-1** fragment is extruded from the nucleus into the cytoplasm) and in TUNEL-negative cells, with only incipient signs of chromatin condensation; this evidence indicates that **PARP-1** degradation in etoposide-treated apoptotic cells may precede DNA cleavage.
- L9 ANSWER 17 OF 17 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation on STN
 2001:594877 The Genuine Article (R) Number: 455HF. Genomic instability in a **PARP-1**(-/-) cell line expressing **PARP-1** DNA-binding domain. Cayuela M L; Carrillo A; Ramirez P;

Parrilla P; Yelamos J (Reprint). Hosp Univ Virgen Arrixaca, Serv Cirugia, Unidad Trasplante, Murcia 30120, Spain (Reprint). BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS (13 JUL 2001) Vol. 285, No. 2, pp. 289-294. ISSN: 0006-291X. Publisher: ACADEMIC PRESS INC, 525 B ST, STE 1900, SAN DIEGO, CA 92101-4495 USA. Language: English.

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Poly(ADP-ribose) polymerase-1 (**PARP-1**) is a nuclear DNA binding protein that participates in processes involving nicking and resealing DNA strands. A genomically unstable subpopulation of **PARP-1**(-/-) cells has recently been described, which disappears after stable transfection of the cells with complete **PARP-1** cDNA. Here we investigate the role played by **PARP-1** in the maintenance of genomic stability, independently of its enzymatic activity. We used a **PARP-1**-deficient cell line to express a DNA construct encoding the **PARP-1** DNA-binding domain (DBD) fragment and one encoding the mutant DBDbd-, defective in binding to DNA strand breaks. We found that, in the absence of DNA damage, expression of DBD or DBDbd-mutant induces increased genomic instability in the **PARP1**(-/-) cells. These results suggest that the DBD fragment of **PARP-1**, apart from its classical role of nick **detection** and DNA binding, is likely to participate in molecular complexes with proteins involved in genomic integrity. (C) 2001 Academic Press.

=> s anti-PARP-1

L10 7 ANTI-PARP-1

=> dup remove l10

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L11 3 DUP REMOVE L10 (4 DUPLICATES REMOVED)

=> d l11 1-3 chib abs

L11 ANSWER 1 OF 3 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN 2005:217426 Document No.: PREV200510007018. Roscovitine-induced up-regulation of p53AIP1 protein precedes the onset of apoptosis in human MCF-7 breast cancer cells (vol 4, pg 113, 2005). Wesierska-Gadek, J.; Gueorguieva, M.; Horky, M.. Molecular Cancer Therapeutics, (MAR 2005) Vol. 4, No. 3, pp. 503. ISSN: 1535-7163. Language: English.

L11 ANSWER 2 OF 3 CAPLUS COPYRIGHT 2005 ACS on STN

2004:20982 Document No. 140:90312 A method for the detection of apoptosis via determination of nucleolin and/or PARP-1 in the sample. Bates, Paula J.; Mi, Yingchang (University of Louisville Research Foundation, Inc., USA). PCT Int. Appl. WO 2004003554 A1 20040108, 66 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, UZ, VC, VN, YU, ZA, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2003-US20167 20030626. PRIORITY: US 2002-2002/PV392143 20020626.

AB Methods for the detection of apoptosis by measuring apoptotic bodies shed by apoptotic cells are provided, as are kits to carry out such methods. The method comprises preparing a sample from which cells have been removed and determining at least one of nucleolin and PARP-1 in the sample. The detection of either (or both) compds. comprises the detection of a nucleolin (PARP-1)-binding mol.-nucleolin (PARP-1) complex. The binding mols. are anti-nucleolin (**anti-PARP-1**) antibodies and for nucleolin also guanosine-rich oligonucleotides. The sample can be blood, serum, plasma, tissue, tissue culture medium, or

sputum. The method can be used to determine excessive apoptosis via preparing

a

blood sample from a subject suspected of having a disease selected from the group consisting of AIDS, neurodegenerative disease, ischemic injury, autoimmune disease, tumor, cancer, viral infection, acute inflammation, and sepsis.

L11 ANSWER 3 OF 3 MEDLINE on STN DUPLICATE 1
2001480638. PubMed ID: 11523787. Ionising radiation induces the expression of PARP-1 and PARP-2 genes in Arabidopsis. Doucet-Chabeaud G; Godon C; Brutesco C; de Murcia G; Kazmaier M. (CEA/Cadarache, DSV-DEVM-Laboratoire de Radiobiologie Vegetale, Saint Paul-lez-Durance, France.) Molecular genetics and genomics : MGG, (2001 Aug) 265 (6) 954-63. Journal code: 101093320. ISSN: 1617-4615. Pub. country: Germany: Germany, Federal Republic of. Language: English.

AB By screening for Arabidopsis genes activated by ionising radiation (IR)-induced DNA damage, we have isolated a cDNA hybridising with a 3.2-kb mRNA that accumulates rapidly and strongly in irradiated cell suspensions or whole plants. The cDNA codes for a 110-kDa protein that is highly homologous to the 116-kDa vertebrate poly(ADP-ribose) polymerase (PARP-1). It is recognised by a human anti-PARP-1 antibody, binds efficiently to DNA strand interruptions in vitro, and catalyses DNA damage-dependent (ADP-ribose) polymer synthesis. We have named this protein AtPARP-1. We have also extended our observations to the Arabidopsis app (AtPARP-2) gene, demonstrating for the first time that IR-induced DNA strand interruptions induce rapid and massive accumulation of AtPARP-1 and AtPARP-2 transcripts, whereas dehydration and cadmium preferentially induce the accumulation of AtPARP-2 transcripts. The IR-induced PARP gene expression seen in Arabidopsis is in striking contrast to the post-translational activation of the PARP-1 protein that is associated with genotoxic stress in animal cells. AtPARP-1 transcripts accumulate in all plant organs after exposure to ionising radiation, but this is followed by an increase in AtPARP-1 protein levels only in tissues that contain large amounts of actively dividing cells. This cell-type specific accumulation of AtPARP-1 protein in response to DNA damage is compatible with a role for the AtPARP-1 protein in the maintenance of DNA integrity during replication, similar to the role of "guardian of the genome" attributed to its animal counterpart.

=> s anti-nucleolin

L12 141 ANTI-NUCLEOLIN

=> s l12

L13 141 L12

=> s l12 and apoptosis

L14 12 L12 AND APOPTOSIS

=> dup remove l14

PROCESSING COMPLETED FOR L14

L15 4 DUP REMOVE L14 (8 DUPLICATES REMOVED)

=> d l15 1-4 cbib abs

L15 ANSWER 1 OF 4 CAPLUS COPYRIGHT 2005 ACS on STN

2004:20982 Document No. 140:90312 A method for the detection of **apoptosis** via determination of nucleolin and/or PARP-1 in the sample. Bates, Paula J.; Mi, Yingchang (University of Louisville Research Foundation, Inc., USA). PCT Int. Appl. WO 2004003554 A1 20040108, 66 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, UZ, VC, VN,

YU, ZA, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2.
APPLICATION: WO 2003-US20167 20030626. PRIORITY: US 2002-2002/PV392143 20020626.

AB Methods for the detection of **apoptosis** by measuring apoptotic bodies shed by apoptotic cells are provided, as are kits to carry out such methods. The method comprises preparing a sample from which cells have been removed and determining at least one of nucleolin and PARP-1 in the sample.

The detection of either (or both) compds. comprises the detection of a nucleolin (PARP-1)-binding mol.-nucleolin (PARP-1) complex. The binding mols. are **anti-nucleolin** (anti-PARP-1) antibodies and for nucleolin also guanosine-rich oligonucleotides. The sample can be blood, serum, plasma, tissue, tissue culture medium, or sputum. The method can be used to determine excessive **apoptosis** via preparing a blood sample from a subject suspected of having a disease selected from the group consisting of AIDS, neurodegenerative disease, ischemic injury, autoimmune disease, tumor, cancer, viral infection, acute inflammation, and sepsis.

L15 ANSWER 2 OF 4 MEDLINE on STN DUPLICATE 1
2003049459. PubMed ID: 12559966. Cleavage of nucleolin and argyrophilic nucleolar organizer region associated proteins in **apoptosis** -induced cells. Kito Shinji; Shimizu Katsuhide; Okamura Hirohiko; Yoshida Kaya; Morimoto Hiroyuki; Fujita Michi; Morimoto Yasuhiro; Ohba Takeshi; Haneji Tatsuji. (Department of Histology and Oral Histology, Kyushu Dental College, Kitakyushu, Japan.) Biochemical and biophysical research communications, (2003 Jan 24) 300 (4) 950-6. Journal code: 0372516. ISSN: 0006-291X. Pub. country: United States. Language: English.

AB To investigate the behavior of nuclear proteins in apoptotic cells, we examined the changes in nucleolin and proteins of the nucleolar organizing region during **apoptosis** in human osteoblastic cell lines, Saos-2 and MG63. **Apoptosis** was induced by treatment of these cells with okadaic acid. Proteins prepared from apoptotic cells were subjected to Western blot analysis and a modified Western blot method using silver nitrate. The **anti-nucleolin** antibody recognized the 110-kDa band and the staining intensity of this band decreased in the proteins prepared from the okadaic acid-treated apoptotic cells. The additional band of an 80-kDa was also detected in the proteins prepared from the apoptotic cells. Two major silver nitrate-stained bands, 110-kDa and 37-kDa, were detected among the proteins obtained from control cells. Like the Western blot analysis, the intensity of the 110-kDa silver nitrate-staining band decreased; an 80-kDa band appeared and its staining intensity increased in the lysate from the okadaic acid-treated cells. The signal intensity of the 37-kDa protein did not change in the sample from the apoptotic cells. In a cell-free apoptotic system, the 80-kDa protein was also detected and the amount of the 110-kDa protein decreased in the extract of Saos-2 cell nuclei incubated with apoptotic cytosol. The change in nucleolin in Saos-2 cells induced to undergo **apoptosis** was examined by an immunocytochemical procedure using the **anti-nucleolin** antibody and Hoechst 33342. Nucleolin was visible as dots in nucleoli in the control cells; however, it was not detected in the cells undergoing **apoptosis**. The dual-exposure view of Hoechst 33342 and **anti-nucleolin** staining cells confirmed that nucleolin had disappeared from the apoptotic nuclei of Saos-2.

L15 ANSWER 3 OF 4 MEDLINE on STN DUPLICATE 2
2002216144. PubMed ID: 11948683. Nucleolin is a calcium-binding protein. Gilchrist James S C; Abrenica Bernard; DiMario Patrick J; Czubyrt Michael P; Pierce Grant N. (Department of Oral Biology and Physiology, Division of Stroke and Vascular Disease, University of Manitoba, Winnipeg, Manitoba, Canada.) Journal of cellular biochemistry, (2002) 85 (2) 268-78. Journal code: 8205768. ISSN: 0730-2312. Pub. country: United States. Language:

English.

- AB We have purified a prominent 110-kDa protein (p110) from 1.6 M NaCl extracts of rat liver nuclei that appears to bind Ca^{2+} . p110 was originally identified by prominent blue staining with 'Stains-All' in sodium dodecyl sulfate-polyacrylamide gels and was observed to specifically bind ruthenium red and $^{45}\text{Ca}^{2+}$ in nitrocellulose blot overlays. In spin-dialysis studies, purified p110 saturably bound approximately 75 nmol Ca^{2+} /mg protein at a concentration of 1 mM total Ca^{2+} with half-maximal binding observed at 105 μM Ca^{2+} . With purification, p110 became increasingly susceptible to proteolytic (likely autolytic) fragmentation, although most intermediary peptides between 40 and 90 kDa retained "Stains-All", ruthenium red, and $^{45}\text{Ca}^{2+}$ binding. N-terminal sequencing of intact p110 and a 70-kDa autolytic peptide fragment revealed a strong homology to nucleolin. Two-dimensional sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE)/IEF revealed autolysis produced increasingly acidic peptide fragments ranging in apparent pI 's from 5.5 for intact p110 to 3.5 for a 40 kDa peptide fragment. Intact p110 and several peptide fragments were immunostained with a highly specific **anti-nucleolin** antibody, R2D2, thus confirming the identity of this protein with nucleolin. These annexin-like Ca^{2+} -binding characteristics of nucleolin are likely contributed by its highly acidic argyrophilic N-terminus with autolysis apparently resulting in largely selective removal of its basic C-terminal domain. Although the Ca^{2+} -dependent functions of nucleolin are unknown, we discuss the possibility that like the structurally analogous HMG-1, its Ca^{2+} -dependent actions may regulate chromatin structure, possibly during **apoptosis**.
Copyright 2002 Wiley-Liss, Inc.

L15 ANSWER 4 OF 4 CAPLUS COPYRIGHT 2005 ACS on STN

2001:161702 Document No. 134-205322 Protein phosphatases and **apoptosis**: nuclear protein associates with protein phosphatase type 1 δ isoform. Haneji, Tatsuji (Second Dep. Oral Anat., Sch. Dent., The Univ. Tokushima, Tokushima 770-8504, Japan). Shikoku Shigakkai Zasshi, 13(2), 221-229 (Japanese) 2001. CODEN: SSZAED. ISSN: 0914-6091. Publisher: Shikoku Shigakkai.

- AB A review with 63 refs. Several lines of evidence indicate that protein phosphorylation and dephosphorylation have been recognized as a key mechanism in cell proliferation, differentiation, and **apoptosis** in various tissues. Okadaic acid is a toxic polyether fatty acid produced by dinoflagellates and is a potent inhibitor of protein phosphatase type 1 (PP1) and type 2A (PP2A) that dephosphorylate serine and threonine residues in eukaryotic cells. cDNA cloning revealed the existence of four isoforms of PP1 catalytic subunit in rat, termed PP1 α , PP1 γ 1, PP1 γ 2, and PP1 δ . PP1 targeting subunits is thought to localize to specific subcellular component and to modulate the activity of the enzyme at these sites. Nucleolin is an abundantly expressed nucleolar phosphoprotein and is located mainly in nucleolus. Staining pattern of nucleolin in MG63 cells is similar to that of the PP1 δ . The dual fluorescence image revealed that PP1 δ and nucleolin represent same localization in nucleolus. The **anti-nucleolin** antibody interacted with the 100 kDa protein immunoprecipitated with PP1 δ antibody. However, **anti-nucleolin** antibody did not interact with the samples precipitated with the normal rabbit serum. The 100

kDa

protein was dephosphorylated into 98 kDa protein by lambda phosphatase. In the actinomycin D-treated cells, subcellular localization of PP1 δ and nucleolin was changed. The amount of PP1 δ increased whereas the level of dephosphorylated form of nucleolin increased. These results indicate that PP1 δ associate with nucleolin directly to dephosphorylate this protein and is involved in r-RNA synthesis. In the present review, I also demonstrate the relationship between protein phosphatases and apoptotic processes in cells including Saos-2, MG63, MC3T3-E1, SCC-25, SCKN, SCTF and HSG cells studied in our laboratory

=> s (bates p?/au or mi y?/au)
L16 2882 (BATES P?/AU OR MI Y?/AU)

=> s l16 and apoptosis
L17 38 L16 AND APOPTOSIS

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PROCESSING COMPLETED FOR L17
L18 14 DUP REMOVE L17 (24 DUPLICATES REMOVED)

=> d l18 1-14 cbib abs

L18 ANSWER 1 OF 14 MEDLINE on STN DUPLICATE 1
2005521504. PubMed ID: 16194912. Establishment of a germ-somatic cell coculture model for toxicity assessment of environmental endocrine disrupters. **Mi Yuling**; Zhang Caiqiao; Zeng Weidong; Tang Xinyan. (College of Animal Sciences, Zhejiang University, Hangzhou, China.) Journal of environmental science and health. Part A, Toxic/hazardous substances & environmental engineering, (2005) 40 (10) 1917-28. Journal code: 9812551. ISSN: 1093-4529. Pub. country: United States. Language: English.
AB The objective of this study was to evaluate the effect of environmental endocrine disrupting chemicals by a germ--somatic cell coculture model in vitro. Testicular cells of 18-day-old chicken embryos were dispersed and cultured in different media. Results showed that somatic cells formed a monolayer to which germ cells adhered in the medium supplemented with insulin (Ins), transferrin (Tf), and selenite (Se) (ITS medium). However, the medium without ITS or single subtraction of Ins, Tf, or Se could not maintain cell survival in culture because many germ cells manifested **apoptosis**. Three known endocrine disrupters were selected to test the feasibility of this model. Aroclor 1254 (A1254, 10 microg/mL) induced condensed nuclei and vacuolated cytoplasm in germ cells, which was further confirmed by a cell proliferation assay. However, after culture for 48 h, the number of germ cells displayed a significant augment stimulated by A1254 (0.1-10 microg/mL) (P < 0.05). Similarly, 2,4-dichlorophenoxyacetic acid and busulfan displayed notable toxic effects on germ cells, and germ cell number and cell viability were significantly decreased in a dose-dependent manner (P < 0.05). The above results indicate that the chicken testicular germ-somatic cell coculture model is a simple, rapid, and veracious in vitro tool for evaluating the effect of environmental endocrine disrupters on functional basis of the cultured cells.

L18 ANSWER 2 OF 14 EMBASE COPYRIGHT (c) 2005 Elsevier B.V. All rights reserved on STN
2005295201 EMBASE TNF- α activates death pathway in human aorta smooth muscle cell in the presence of 7-ketocholesterol. Hyun S.L.; Jong S.C.; Jin A.B.; **Mi Y.C.**; Han C.L.; Byung Y.R.; Dai E.S.; Rho M.-C.; Young K.K.; Kim K.. K. Kim, Department of Pharmacology, College of Medicine, Pusan National University, Pusan, Korea, Republic of. koanhoi@pusan.ac.kr. Biochemical and Biophysical Research Communications Vol. 333, No. 4, pp. 1093-1099 12 Aug 2005.
Refs: 26.
ISSN: 0006-291X. CODEN: BBRCA
S 0006-291X(05)01186-1. Pub. Country: United States. Language: English. Summary Language: English.

ED Entered STN: 20050805

AB This study was undertaken to investigate whether a physiologically compatible concentration of 7-ketocholesterol had any effect on human vascular smooth muscle cells (HVSMCs). We found that 7-ketocholesterol changed the viability of human aorta smooth muscle cells (HAOSMC) not by cytotoxicity but by activation of tumor necrosis factor- α receptor (TNFR)-mediated death. Whereas TNF- α did not affect the viability in the presence of 7 α -hydroxycholesterol or cholesterol, the cytokine induced HAOSMC death in the presence of 7-ketocholesterol as

detected by morphology, viability, and fragmentation of chromosomal DNA. The HAOSMC death was inhibited by a neutralizing anti-TNF receptor 1 (TNFR1) antibody and by the caspase inhibitors of z-VAD and z-DEVD. Activations of caspase-8 and -3 were detected from dying HAOSMCs. 7-Ketocholesterol inhibited translocation of the nuclear factor κ B (NF- κ B) subunits of p65 and p50 from the cytosol into the nucleus, increase of NF- κ B activity, and expression of caspase-8 homolog Fas ligand interleukin-1-converting enzyme inhibitory protein by TNF- α . We also found that X-chromosome-linked inhibitor of **apoptosis** protein was degraded in dying HAOSMC. The present study proposes that 7-ketocholesterol would contribute to the disappearance of HVSMC in the atherosclerotic lesions by enhancing receptor-mediated death. This is the first report demonstrating induction of TNF- α -mediated death by oxysterol in cells. .COPYRGT. 2005 Elsevier Inc. All rights reserved.

L18 ANSWER 3 OF 14 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN 2005:347023 Document No.: PREV200510140181. Nucleolin, a novel target for aptamer-based anticancer therapy. Miller, D. M. [Reprint Author]; **Bates, P.**; Trent, J.; Sharma, V.; Thomas, S.; Barnhart, K.; Laber, D.. Univ Louisville, Sch Med, James Graham Brown Canc Ctr, Louisville, KY 40292 USA. Journal of Investigative Medicine, (JAN 2005) Vol. 53, No. 1, Suppl. S, pp. S305. Meeting Info.: Western Student Medical Research Forum held in conjunction with the Western Section of the American-Federation-for-Medical-Research/Western-Society-for-Clinical-Investigation/Western-Association-of-Physicians/Western-Society-for-Pediatric-Research. Carmel, CA, USA. February 03 -05, 2005. Amer Federat Med Res, Western Sect; Western Soc Clin Investigat; Western Assoc Physicians; Western Soc Pediat Res. ISSN: 1081-5589. Language: English.

L18 ANSWER 4 OF 14 CAPLUS COPYRIGHT 2005 ACS on STN 2004:20982 Document No. 140:90312 A method for the detection of **apoptosis** via determination of nucleolin and/or PARP-1 in the sample. **Bates, Paula J.**; **Mi, Yingchang** (University of Louisville Research Foundation, Inc., USA). PCT Int. Appl. WO 2004003554 A1 20040108, 66 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, UZ, VC, VN, YU, ZA, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2003-US20167 20030626. PRIORITY: US 2002-2002/PV392143 20020626.

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L18 ANSWER 5 OF 14 MEDLINE on STN DUPLICATE 2 2004456047. PubMed ID: 15364206. Effects of follicle-stimulating hormone and androgen on proliferation of cultured testicular germ cells of embryonic chickens. **Mi Yuling**; Zhang Caiqiao; Xie Meina; Zeng Weidong. (Department of Veterinary Medicine, College of Animal Sciences,

Zhejiang University, Hangzhou 310029, China.) General and comparative endocrinology, (2004 Sep 15) 138 (3) 237-46. Journal code: 0370735. ISSN: 0016-6480. Pub. country: United States. Language: English.

- AB A germ-Sertoli cell coculture model was established to study effects of follicle-stimulating hormone (FSH) and testosterone (T) on testicular germ cell proliferation of the embryonic chickens. Germ and somatic cells were dispersed from 18-day-old embryonic testes and cultured in 96-well plates. Germ cells were characterized by expression of stem cell factor receptor c-kit. Germ cell proliferation was assessed by an increase in cell number and expression of proliferating cell nuclear antigen (PCNA). Results showed that the germ and Sertoli cells kept alive in serum-free McCoy's 5A medium supplemented with insulin, transferrin, and selenite (ITS medium). Germ cells adhered to the free surface of Sertoli cells that spread the filopodia and formed a monolayer in ITS medium. In the serum-containing medium, Sertoli cells displayed an increment with a flat squamous form and only a few very large germ cell masses were found in the free surface of Sertoli cells. Many germ cells showed **apoptosis** in the McCoy's 5A medium without ITS or serum. Only germ cells showed positive staining for c-kit in the coculture. Ovine FSH (0.25-1.0 IU/ml) significantly increased the number of germ cells, and PCNA-labeling index ($P < 0.05$). FSH also induced stronger c-kit expression compared with the control. In the FSH-treated groups, germ cells were manifested distinct knob-like form. Similar stimulating effect was found in the germ cell number by T treatments (10(-7)-10(-6)M). Furthermore, FSH (0.5 IU/ml) combined with T significantly promoted higher testicular germ cell proliferation ($P < 0.05$) compared with either FSH or T alone, which indicated that interaction of FSH and T might be additive. The above results showed that the serum-free germ-Sertoli cell coculture model allowed evaluating hormonal regulation of testicular germ cell proliferation. FSH and T promoted testicular germ cell proliferation probably through indirect effects on Sertoli cells.

L18 ANSWER 6 OF 14 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation on STN

2004:250658 The Genuine Article (R) Number: 778LL. Identification of murine T-cell epitopes in Ebola virus nucleoprotein. Simmons G; Lee A; Rennekamp A J; Fan X; Bates P (Reprint); Shen H. Univ Penn, Sch Med, Dept Microbiol, 3610 Hamilton Walk, Philadelphia, PA 19104 USA (Reprint); Univ Penn, Sch Med, Dept Microbiol, Philadelphia, PA 19104 USA. VIROLOGY (5 JAN 2004) Vol. 318, No. 1, pp. 224-230. ISSN: 0042-6822. Publisher: ACADEMIC PRESS INC ELSEVIER SCIENCE, 525 B ST, STE 1900, SAN DIEGO, CA 92101-4495 USA. Language: English.

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

- AB CD8 T cells play an important role in controlling Ebola infection and in mediating vaccine-induced protective immunity, yet little is known about antigenic targets in Ebola that are recognized by CD8 T cells. Overlapping peptides were used to identify major histocompatibility complex class I-restricted epitopes in mice immunized with vectors encoding Ebola nucleoprotein (NP). CD8 T-cell responses were mapped to a H-2(d)-restricted epitope (NP279-288) and two H-2(b)-restricted epitopes (NP44-52 and NP288-296). The identification of these epitopes will facilitate studies of immune correlates of protection and the evaluation of vaccine strategies in murine models of Ebola infection. (C) 2003 Elsevier Inc. All rights reserved.

L18 ANSWER 7 OF 14 MEDLINE on STN DUPLICATE 3

2003099862. PubMed ID: 12506112. **Apoptosis** in leukemia cells is accompanied by alterations in the levels and localization of nucleolin. Mi Yingchang; Thomas Shelia D; Xu Xiaohua; Casson Lavona K; Miller Donald M; Bates Paula J. (Molecular Targets Group, James Graham Brown Cancer Center, Department of Medicine, University of Louisville, Kentucky 40202, USA.) Journal of biological chemistry, (2003 Mar 7) 278 (10) 8572-9. Electronic Publication: 2002-12-27. Journal code: 2985121R. ISSN: 0021-9258. Pub. country: United States. Language: English.

- AB Molecular defects in apoptotic pathways are thought to often contribute to

the abnormal expansion of malignant cells and their resistance to chemotherapy. Therefore, a comprehensive knowledge of the mechanisms controlling induction of **apoptosis** and subsequent cellular disintegration could result in improved methods for prognosis and treatment of cancer. In this study, we have examined **apoptosis**-induced alterations in two proteins, nucleolin and poly(ADP-ribose) polymerase-1 (PARP-1), in U937 leukemia cells. Nucleolin is expressed at high levels in malignant cells, and it is a multifunctional and mobile protein that can shuttle among the nucleolus, nucleoplasm, cytoplasm, and plasma membrane. Here, we report our findings that UV irradiation or camptothecin treatment of U937 cells induced **apoptosis** and caused a significant change in the levels and localization of nucleolin within the nucleus. Additionally, nucleolin levels were dramatically decreased in extracts containing the cytoplasm and plasma membrane. These alterations could be abrogated by pre-incubation with an inhibitor of PARP-1 (3-aminobenzamide), and our data support a potential role for nucleolin in removing cleaved PARP-1 from dying cells. Furthermore, both nucleolin and cleaved PARP-1 were detected in the culture medium of cells undergoing **apoptosis**, associated with particles of a size consistent with apoptotic bodies. These results indicate that nucleolin plays an important role in **apoptosis**, and could be a useful marker for assessing **apoptosis** or detecting apoptotic bodies. In addition, the data provide a possible explanation for the appearance of nucleolin and PARP-1 autoantibodies in some autoimmune diseases.

L18 ANSWER 8 OF 14 MEDLINE on STN DUPLICATE 4
 2003165071. PubMed ID: 12682360. Biophysical and biological properties of quadruplex oligodeoxyribonucleotides. Dapic Virna; Abdomerovic Vedra; Marrington Rachel; Peberdy Jemma; Rodger Alison; Trent John O; **Bates Paula J.** (Department of Medicine, James Graham Brown Cancer Center, University of Louisville, Louisville, KY 40202, USA.) Nucleic acids research, (2003 Apr 15) 31 (8) 2097-107. Journal code: 0411011. ISSN: 1362-4962. Pub. country: England: United Kingdom. Language: English.

AB Single-stranded guanosine-rich oligodeoxyribonucleotides (GROs) have a propensity to form quadruplex structures that are stabilized by G-quartets. In addition to intense speculation about the role of G-quartet formation in vivo, there is considerable interest in the therapeutic potential of quadruplex oligonucleotides as aptamers or non-antisense antiproliferative agents. We previously have described several GROs that inhibit proliferation and induce **apoptosis** in cancer cell lines. The activity of these GROs was related to their ability to bind to a specific cellular protein (GRO-binding protein, which has been tentatively identified as nucleolin). In this report, we describe the physical properties and biological activity of a group of 12 quadruplex oligonucleotides whose structures have been characterized previously. This group includes the thrombin-binding aptamer, an anti-HIV oligonucleotide, and several quadruplexes derived from telomere sequences. Thermal denaturation and circular dichroism (CD) spectropolarimetry were utilized to investigate the stability, reversibility and ion dependence of G-quartet formation. The ability of each oligonucleotide to inhibit the proliferation of cancer cells and to compete for binding to the GRO-binding protein was also examined. Our results confirm that G-quartet formation is essential for biological activity of GROs and show that, in some cases, quadruplex structures formed in the presence of potassium ions are significantly more active than those formed in the presence of sodium ions. However, not all quadruplex structures exhibit antiproliferative effects, and the most accurate factor in predicting biological activity was the ability to bind to the GRO-binding protein. Our data also indicate that the CD spectra of quadruplex oligonucleotides may be more complex than previously thought.

L18 ANSWER 9 OF 14 MEDLINE on STN DUPLICATE 5
 2001652572. PubMed ID: 11555643. Inhibition of DNA replication and induction of S phase cell cycle arrest by G-rich oligonucleotides. Xu X; Hamhouyia F; Thomas S D; Burke T J; Girvan A C; McGregor W G; Trent J O;

Miller D M; Bates P J. (Human Molecular Biology Group, James Graham Brown Cancer Center, Department of Medicine, University of Louisville, Louisville, Kentucky 40202, USA.) Journal of biological chemistry, (2001 Nov 16) 276 (46) 43221-30. Electronic Publication: 2001-09-12. Journal code: 2985121R. ISSN: 0021-9258. Pub. country: United States. Language: English.

AB The discovery of G-rich oligonucleotides (GROs) that have non-antisense antiproliferative activity against a number of cancer cell lines has been recently described. This biological activity of GROs was found to be associated with their ability to form stable G-quartet-containing structures and their binding to a specific cellular protein, most likely nucleolin (Bates, P. J., Kahlon, J. B., Thomas, S. D., Trent, J. O., and Miller, D. M. (1999) J. Biol. Chemical 274, 26369-26377). In this report, we further investigate the novel mechanism of GRO activity by examining their effects on cell cycle progression and on nucleic acid and protein biosynthesis. Cell cycle analysis of several tumor cell lines showed that cells accumulate in S phase in response to treatment with an active GRO. Analysis of 5-bromodeoxyuridine incorporation by these cells indicated the absence of de novo DNA synthesis, suggesting an arrest of the cell cycle predominantly in S phase. At the same time point, RNA and protein synthesis were found to be ongoing, indicating that arrest of DNA replication is a primary event in GRO-mediated inhibition of proliferation. This specific blockade of DNA replication eventually resulted in altered cell morphology and induction of **apoptosis**. To characterize further GRO-mediated inhibition of DNA replication, we used an in vitro assay based on replication of SV40 DNA. GROs were found to be capable of inhibiting DNA replication in the in vitro assay, and this activity was correlated to their antiproliferative effects. Furthermore, the effect of GROs on DNA replication in this assay was related to their inhibition of SV40 large T antigen helicase activity. The data presented suggest that the antiproliferative activity of GROs is a direct result of their inhibition of DNA replication, which may result from modulation of a replicative helicase activity.

L18 ANSWER 10 OF 14 EMBASE COPYRIGHT (c) 2005 Elsevier B.V. All rights reserved on STN

2001367515 EMBASE Cisplatin-induced **apoptosis** by translocation of endogenous Bax in mouse collecting duct cells. Ryang Hwa Lee; Jin Mi Song; **Mi Young Park**; Soo Kyung Kang; Yong Keun Kim; Jing Sup Jung. J.S. Jung, College of Medicine, Pusan National University, 1 Ga, Ami-Dong, Suh-Gu, Pusan (602-739), Korea, Republic of. jsjung@hyowon.pusan.ac.kr. Biochemical Pharmacology Vol. 62, No. 8, pp. 1013-1023 1 Nov 2001. Refs: 60.

ISSN: 0006-2952. CODEN: BCPCA6

S 0006-2952(01)00748-1. Pub. Country: United States. Language: English. Summary Language: English.

ED Entered STN: 20011102

AB cis-Platinum(II) (cis-diammine dichloroplatinum; cisplatin) is a potent antitumor compound that is widely used for the treatment of many malignancies. An important side-effect of cisplatin is nephrotoxicity, which results from injury to renal tubular epithelial cells and can be manifested as either acute renal failure or a chronic syndrome characterized by renal electrolyte wasting. Recently, **apoptosis** has been recognized as an important mechanism of cell death mediating the antitumor effect of cisplatin. This study was undertaken to examine the mechanisms of cell death induced by cisplatin in M-1 cells, which were derived from the outer cortical collecting duct cells of SV40 transgenic mice. Treatment of M-1 cells with high concentrations of cisplatin (0.5 and 1 mM) for 2 hr led to necrotic cell death, whereas a 24-hr treatment with 5-20 μ M cisplatin led to **apoptosis**. Antioxidants protected against cisplatin-induced necrosis, but not **apoptosis**, indicating that reactive oxygen species play a role in mediating necrosis but not **apoptosis** induced by cisplatin and that the mechanism of cell death induced by cisplatin is concentration dependent. The low concentrations of cisplatin, which induced **apoptosis** in M-1

cells, did not affect the expression levels of Bcl-2-related proteins and did not activate c-Jun NH(2)-terminal kinase (SAPK/JNK). Cisplatin induced the translocation of endogenous Bax from the cytosolic to the membrane fractions and, subsequently, the release of cytochrome c. Overexpression of Bcl-2 blocked cisplatin-induced **apoptosis** and Bax translocation. These observations suggest that the subcellular redistribution of Bax is a critical event in the **apoptosis** induced by cisplatin. .COPYRG. 2001 Elsevier Science Inc. All rights reserved.

L18 ANSWER 11 OF 14 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN

2002:151933 Document No.: PREV200200151933. Regulation of nucleolin in U937 cells treated with UV-light and cytotoxic drugs. **Mi, Yingchang** [Reprint author]; Rates, Paula J. [Reprint author]; Thomas, Shelia D. [Reprint author]; Xu, Xiaohua [Reprint author]; Casson, Lavona [Reprint author]; Miller, Donald M. [Reprint author]. James Graham Brown Cancer Center, University of Louisville, Louisville, KY, USA. Blood, (November 16, 2001) Vol. 98, No. 11 Part 2, pp. 139b. print. Meeting Info.: 43rd Annual Meeting of the American Society of Hematology, Part 2. Orlando, Florida, USA. December 07-11, 2001. American Society of Hematology.

CODEN: BLOOAW. ISSN: 0006-4971. Language: English.

AB Levels of the nucleolar protein, nucleolin, are positively correlated with cell proliferation rate, and therefore elevated in cancer cells compared to normal cells. Nucleolin is a multifunctional protein that has been implicated in many processes, including ribosome biogenesis, DNA replication, cell cycle progression and **apoptosis**. It has been identified as an **apoptosis**-associated protein in human Burkitt lymphoma cell line, and is cleaved by caspase-3. Poly(ADP-ribose) polymerase (PARP) is an enzyme involved in DNA repair and one of intracellular "death substrates", can also be cleaved by caspase-3. In this study, U937 (monocytic leukemia) cells were irradiated with UV-light or treated with 20 mM cytosine arabinoside (Ara-C), 10 mM camptothecin (CPT) for different times (2, 4, 8, 24 h) in the absence or presence of 3-aminobenzamide (3-ABA), an inhibitor of PARP. Alterations of nucleolin in both cytoplasm and nucleus, and PARP in nucleus were investigated by western blot analysis. Cell cycle parameters were determined using flow cytometry with PI staining. Nucleolin in the cytoplasm decreased 4h after UV-irradiation and did not come back until 72h. Nucleolin in the nucleus decreased 2h after UV-irradiation, and recovered 48h later. The active form of PARP (118-KD protein) began cleavage to an inactive form (89 Kda) 2h after UV-irradiation, became undetectable at 4h, and came back at 48h. 3-ABA pre-incubation could inhibit PARP cleavage by more than 50% at 4h, 8h, 24h. At the same time, 3-ABA also reduced the disappearance of nucleolin (both in cytoplasm and nucleus). We also compared cell cycle after UV-irradiation between 3-ABA pre-incubated and no 3-ABA treatment groups. Percentage of sub-G1 phase cells was the highest at 4h, and decreased gradually. The 3-ABA pre-incubated group had a higher percentage of S phase cells and a lower ratio of sub-G1 phase cells. Furthermore, we treated U937 cells using Ara-C and CPT, and found that nucleolin both in cytoplasm and nucleus was down-regulated over time and decreased markedly by 8h. PARP was cleaved to its inactive form at 4h. Although pre-incubation with 3-ABA before drug treatment did not protect PARP cleavage, it protected nucleolin from decreasing (although to a lesser extent than in UV-irradiated cells). Using immunoprecipitation/western blot we determined that nucleolin and PARP could form a complex. Conclusions: Nucleolin plays an important role in leukemia cell **apoptosis**/death induced by anti-neoplastic agents and UV-irradiation. Alteration of nucleolin in nucleus precludes that in the cytoplasm. Nucleolin can form a complex with PARP and acts as one of the substrates of PARP. Nucleolin is therefore a component of the caspase-dependent cell **apoptosis** mechanism.

L18 ANSWER 12 OF 14 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on

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2001:301451 Document No.: PREV200100301451. Guanosine-rich oligonucleotides inhibit proliferation of leukemia cells. Castillos, Francisco A., III [Reprint author]; Bates, Paula J. [Reprint author]; Thomas, Shelia D. [Reprint author]; Xu, Xiaohua [Reprint author]; Trent, John O. [Reprint author]; Miller, Donald M. [Reprint author]. Hematology/Medical Oncology James Graham Brown Cancer Center, University of Louisville, Louisville, KY, USA. Blood, (November 16, 2000) Vol. 96, No. 11 Part 1, pp. 308a. print.
Meeting Info.: 42nd Annual Meeting of the American Society of Hematology. San Francisco, California, USA. December 01-05, 2000. American Society of Hematology.

CODEN: BLOOAW. ISSN: 0006-4971. Language: English.

AB We have tested the ability of a G-quartet forming Guanosine-rich oligonucleotide GRO29A to inhibit growth of several leukemia cell-lines in vitro. GRO29A is a novel oligonucleotide which has its effects by non-antisense mechanisms. MTT assays were performed to determine dose-response to GRO29A in U937, K562, HL-60, MEG01, and RS4:11 leukemia cell-lines and the mouse hematopoietic progenitor stem-cell line ATCC 2037. We demonstrated IC50s ranging from 2.0 to 2.5 μ M for leukemia cell-lines with differential survival of a population of mouse hematopoietic progenitor cells in the 10 μ M range. Time course assays demonstrate a sustained inhibition of growth at 96 hrs after a 48 hr washout period of GRO29A-treated U937 cells compared to U937 PBS-treated control cells. We further characterized the effect of GRO29A on leukemia cell-lines using cell cycle analysis by flow cytometry, which demonstrated apprx60% increase in S-phase cells with a concomitant decrease in G0/G1, and total loss of G2/M phase cells treated with (GRO29A) at gtoreq 1 μ M. There was also a subG0/G1 peak in U937 cells treated with (GRO29A) at gtoreq 1 μ M. Apoptosis was confirmed by TUNEL assay in GRO29A-treated U937 cells in time course assays with a prolonged 14% increase over PBS-treated control cells occurring 28 hours after initial GRO29A exposure. Colony formation is inhibited 100% at gtoreq 1 X 10.6 M log (GRO29A) consistent with the dose-response results. The inhibition of growth induced by GRO29A correlates with its binding to specific protein bands at the same molecular weight in all cell-lines tested by southwestern analysis. One of these bands corresponds to nucleolin antibody staining of the same blot by western analysis. Using electrophoretic mobility shift assays we detected a nuclear protein that binds specifically to GRO29A and this same nuclear protein is bound by the telomere sequence. Levels of protein binding from nuclear extracts of these leukemia cell-lines correlates with inhibition of cell growth by GRO29A. We are investigating the relationship between this inhibition of proliferation by GRO29A in these leukemia cell-lines and the ability of GRO29A to competitively inhibit nucleolin/human DNA helicase IV using helicase assays. These results provide a new therapeutic target for the treatment of leukemias.

L18 ANSWER 13 OF 14 MEDLINE on STN

DUPLICATE 6

2001554558. PubMed ID: 11601276. Effect of WT1 gene expression on cell growth and proliferation in myeloid leukemia cell lines. Mi Y; Wang L; Bian S; Meng Q; Chen G; Wang J. (Institute of Hematology and Blood Diseases Hospital, CAMS, PUMC, Tianjin 300020, China.) Chinese medical journal, (1999 Aug) 112 (8) 705-8. Journal code: 7513795. ISSN: 0366-6999. Pub. country: China. Language: English.

AB OBJECTIVE: To investigate the effects and mechanism of Wilms' tumor (WT1) antisense oligonucleotides (AS-oligomers) on proliferation and apoptosis in myeloid leukemia cell lines. METHODS: K562 and HL-60 cells were cultured in presence of WT1 oligomers. Both cell lines express WT1 gene with no p53 protein expression. Cells growth, apoptosis and expression of WT1, bcl-2 genes were analysed using 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenylmetrazolium bromide (MTT) colorimetric assay, flow cytometry and reverse transcription-polymerase chain reaction (RT-PCR) methods. RESULTS: WT1 antisense oligonucleotides inhibited cellular proliferation of K562 cells and the effect was

concentration-dependent. When cultured at concentration of 200 micrograms/ml oligomers, growth inhibition was 46.2% for antisense oligonucleotide cultivated group and 28.1% for sense oligonucleotide cultured group (P = 0.008) respectively. WT1 antisense oligonucleotide can induce **apoptosis** of K562 and HL-60 cells. Percentages of apoptotic cells in antisense oligonucleotide and sense oligonucleotide treated groups were 30.88% versus 13.62% for K562 cells and 40.15% versus 4.23% for HL-60 cells. However the growth of HL-60 cells and expression of bcl-2 gene were unaffected. CONCLUSIONS: The WT1 gene is related with proliferation and **apoptosis** of leukemic cells. Effect of anti-**apoptosis** may be independent of the cellular p53 status and bcl-2 expression. WT1 gene may play an important role in leukemogenesis.

L18 ANSWER 14 OF 14 MEDLINE on STN DUPLICATE 7
2001162927. PubMed ID: 11263329. Effect of WT1 gene expression on cell growth and proliferation in myeloid leukemia cell lines. **Mi Y;** Wang L; Bian S. (Institute of Hematology and Blood Diseases Hospital, CAMS and PUMC, Tianjin 300020.) Zhonghua xue ye xue za zhi = Zhonghua xueyexue zazhi, (1998 Dec) 19 (12) 627-30. Journal code: 8212398. ISSN: 0253-2727. Pub. country: China. Language: Chinese.

AB OBJECTIVE: To explore the effect of WT1 antisense oligonucleotide(AS-oligo) on cell proliferation and **apoptosis** in myeloid leukemia cell lines. METHODS: K562 and HL-60 cells were cultivated with WT1 AS-oligo. The cell proliferation, **apoptosis**, cell cycle and gene expression were examined by MTT colorimetry, FACS and RT-PCR. RESULTS: WT1 AS-oligo could inhibit the proliferation of K562 cell and induce **apoptosis** of K562 and HL-60 cells. On the contrary, the growth of HL-60 cells and the expression of WT1, mdm2 and bcl-2 genes were unaffected. CONCLUSION: WT1 gene is related to the proliferation and **apoptosis** of leukemic cells. WT1 gene could suppress cell **apoptosis** independent of status of p53 and bcl-2 genes. It might play an role in leukemogenesis.

=> s l16 and anti-nucleolin
L19 1 L16 AND ANTI-NUCLEOLIN

=> d l19 chib abs

L19 ANSWER 1 OF 1 CAPLUS COPYRIGHT 2005 ACS on STN
2004:20982 Document No. 140:90312 A method for the detection of apoptosis via determination of nucleolin and/or PARP-1 in the sample. **Bates, Paula J.; Mi, Yingchang** (University of Louisville Research Foundation, Inc., USA). PCT Int. Appl. WO 2004003554 A1 20040108, 66 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, UZ, VC, VN, YU, ZA, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2003-US20167 20030626. PRIORITY: US 2002-2002/PV392143 20020626.

AB Methods for the detection of apoptosis by measuring apoptotic bodies shed by apoptotic cells are provided, as are kits to carry out such methods. The method comprises preparing a sample from which cells have been removed and determining at least one of nucleolin and PARP-1 in the sample. The detection of either (or both) compds. comprises the detection of a nucleolin (PARP-1)-binding mol.-nucleolin (PARP-1) complex. The binding mols. are **anti-nucleolin** (anti-PARP-1) antibodies and for nucleolin also guanosine-rich oligonucleotides. The sample can be blood, serum, plasma, tissue, tissue culture medium, or sputum. The method can be used to determine excessive apoptosis via preparing a blood sample

from a subject suspected of having a disease selected from the group consisting of AIDS, neurodegenerative disease, ischemic injury, autoimmune disease, tumor, cancer, viral infection, acute inflammation, and sepsis.

=> s l16 and anti-PARP
L20 1 L16 AND ANTI-PARP

=> d l20 chib abs

L20 ANSWER 1 OF 1 CAPLUS COPYRIGHT 2005 ACS on STN
2004:20982 Document No. 140:90312 A method for the detection of apoptosis via determination of nucleolin and/or PARP-1 in the sample. **Bates, Paula J.; Mi, Yingchang** (University of Louisville Research Foundation, Inc., USA). PCT Int. Appl. WO 2004003554 A1 20040108, 66 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, UZ, VC, VN, YU, ZA, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2003-US20167 20030626. PRIORITY: US 2002-2002/PV392143 20020626.

AB Methods for the detection of apoptosis by measuring apoptotic bodies shed by apoptotic cells are provided, as are kits to carry out such methods. The method comprises preparing a sample from which cells have been removed and determining at least one of nucleolin and PARP-1 in the sample. The detection of either (or both) compds. comprises the detection of a nucleolin (PARP-1)-binding mol.-nucleolin (PARP-1) complex. The binding mols. are anti-nucleolin (**anti-PARP-1**) antibodies and for nucleolin also guanosine-rich oligonucleotides. The sample can be blood, serum, plasma, tissue, tissue culture medium, or sputum. The method can be used to determine excessive apoptosis via preparing a blood sample from a subject suspected of having a disease selected from the group consisting of AIDS, neurodegenerative disease, ischemic injury, autoimmune disease, tumor, cancer, viral infection, acute inflammation, and sepsis.

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Purification: unpurified

Clonal type: monoclonal

Ig Type: IgG

Host: mouse

Immunogen: Nuclear matrix extract from MDCK cells

Recognizes: nucleolin

Molecular Weight: 100kDa

Quality Assurance:
routinely evaluated by immunoblot on HeLa nuclear extract

Stability: 2 years at -20°C

GenBank Accession # (click for details from Entrez Gene):
[NM_005381](#)

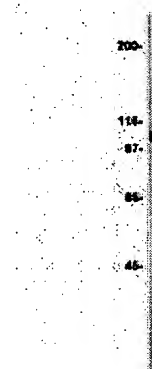
Tested Applications: Western blotting, immunoprecipitation, immunocytochemistry

Species Reactivity: human, monkey, canine

General reviews of this protein:
Gotzmann, J., et al., Electrophoresis 18: 2645, 1997

Published citations in which this product was used:
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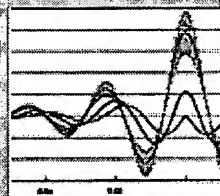
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Antigen	Nucleolin
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Product Description

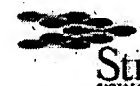
This antibody detects an ~100 kDa protein, corresponding to the apparent molecular mass of nucleolin on SDS-PAGE in This antibody allows in situ detection of nucleolar protein in cells and paraffin embedded tissue sections.

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B23 (H-106)	sc-5564	rabbit IgG	174-280 (h)	WB, IP, IF
NPM3 (N-15)	sc-21474	goat IgG	N-terminus (h)	WB
C23 (H-250)	sc-13057	rabbit IgG	271-520 (h)	WB, IP, IF
NPM3 (C-13)	sc-21476	goat IgG	within an internal region (h)	WB
B23 (C-19)	sc-6013	goat IgG	C-terminus (h)	WB, IP, IF, IHC

Product	Cat.#	Description	Application
C23 (271-520)	sc-4443	WB Protein	271-520 (h) Positive Control

Product	Cat.#	Isotype	Epitope	Application
B23 (FC-8791)	sc-32256	mouse IgG ₁	epitope corresponding to the C-terminal 68 amino acids	WB, IP, IF

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Transcription Regulators: C23/Nucleolin and B23

C23, also designated nucleolin, is a nucleolar protein that is involved in ribosome biogenesis. C23 contains domains, which interact with pre-RNA during synthesis. C23 influences pre-RNA processing, regulation of transcription and nucleolar targeting of ribosomal components. Phosphorylation by Cdc2 and casein kinase translocation of C23 from the nucleolus to the cytoplasm. B23, also designated nucleophosmin, is an abundant phosphoprotein with a high affinity for peptides containing nuclear localization signals. B23 participates in rNPM3 is a nuclear protein that is related to the nuclear chaperone phosphoproteins, nucleoplasmin and nucleolin, which are highly expressed in the pancreas and testis.

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Product	Cat.#	Isotype	Epitope	Application
C23 (F-18)	sc-9893	goat IgG	internal (h)	WB, IP, IF, IHC

Additional C23/Nucleolin and B23 and related products: (Results **1 - 10** of **10**)

Product	Cat.#	Isotype	Epitope	Application
C23 (D-6)	sc-17826	mouse IgG _{2a}	271-520 (h)	WB, IP, IF
B23 (H-106)	sc-5564	rabbit IgG	174-280 (h)	WB, IP, IF
C23 (MS-3)	sc-8031	mouse IgG ₁	1-706 (h)	WB, IP, IF, IHC
NPM3 (N-15)	sc-21474	goat IgG	N-terminus (h)	WB
C23 (H-250)	sc-13057	rabbit IgG	271-520 (h)	WB, IP, IF
NPM3 (C-13)	sc-21476	goat IgG	within an internal region (h)	WB
B23 (C-19)	sc-6013	goat IgG	C-terminus (h)	WB, IP, IF, IHC

Product	Cat.#	Description	
C23 (271-520)	sc-4443	WB Protein	271-520 (h) Positive Control

Product	Cat.#	Isotype	Epitope	Application
B23 (FC-8791)	sc-32256	mouse IgG ₁	epitope corresponding to the C-terminal 68 amino acids	WB, IP, IF